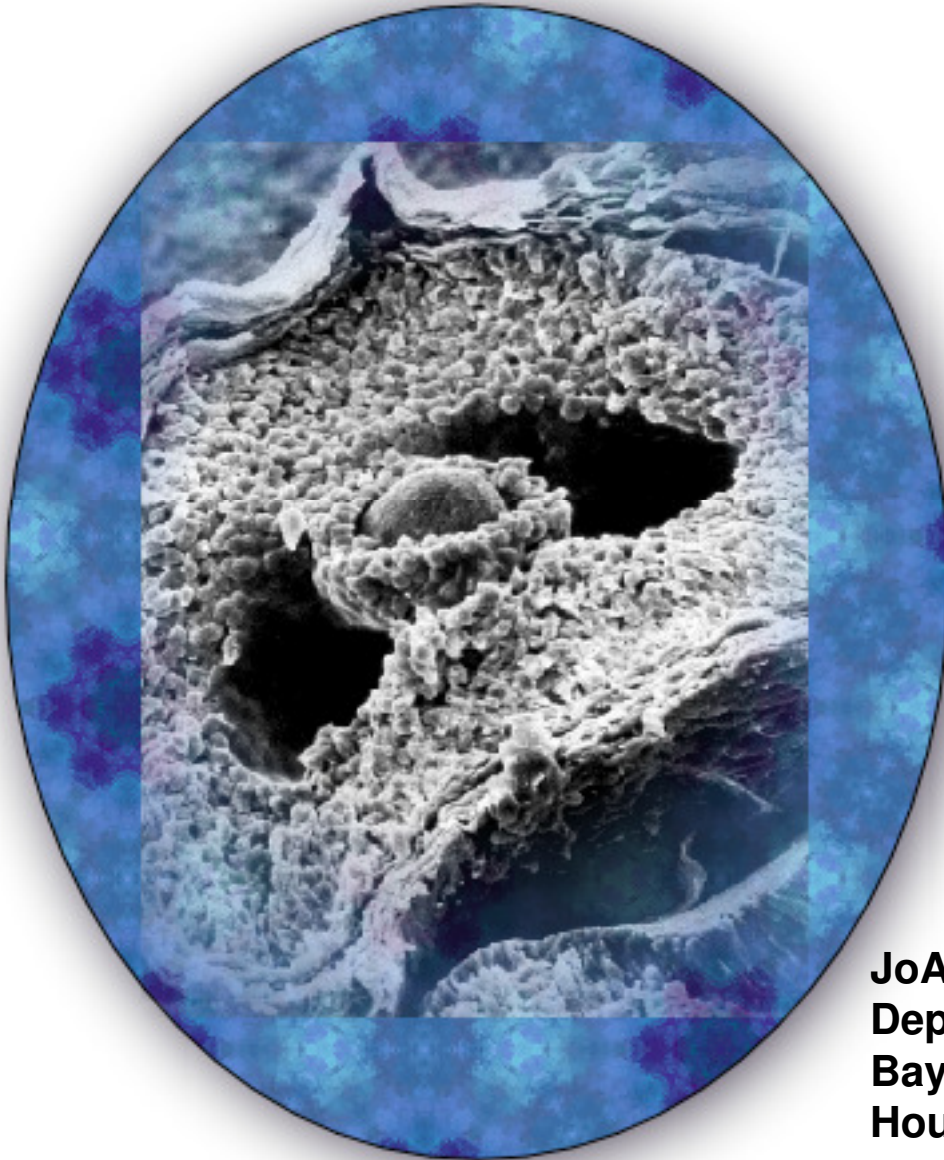


Immune-like Mechanisms Associated with Ovulation:



Inflammatory reactions

Matrix remodeling

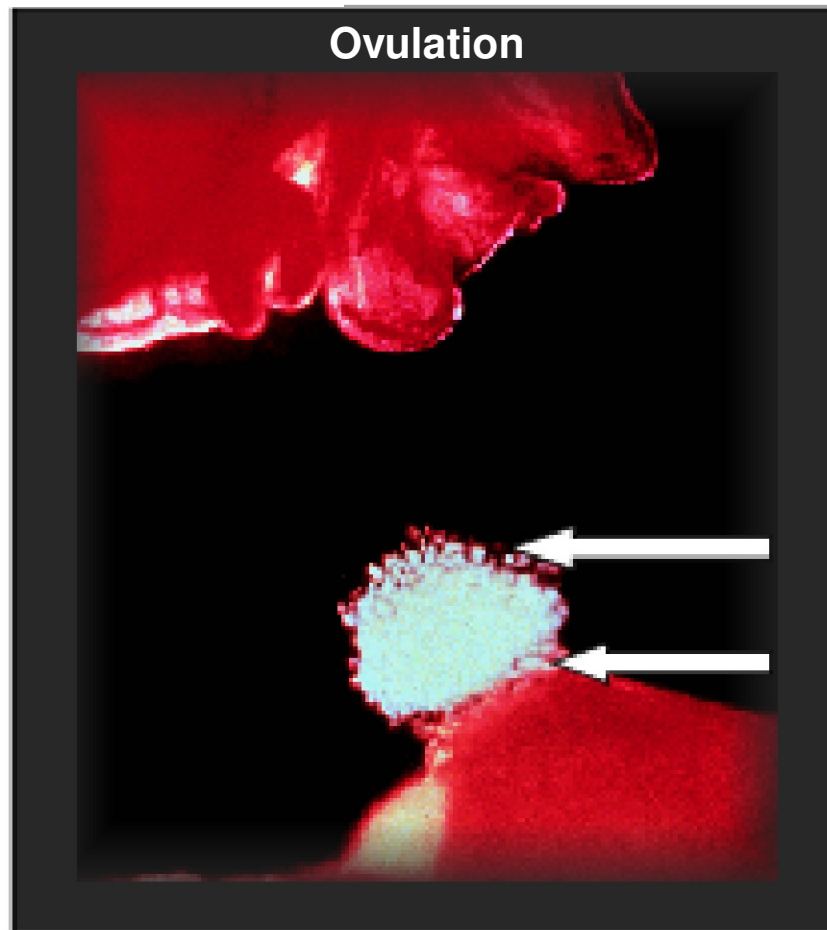
**Cytokine production
and actions**

Innate immune genes

JoAnne S. Richards, PhD
Department of Molecular and Cellular Biology
Baylor College of Medicine
Houston, Texas

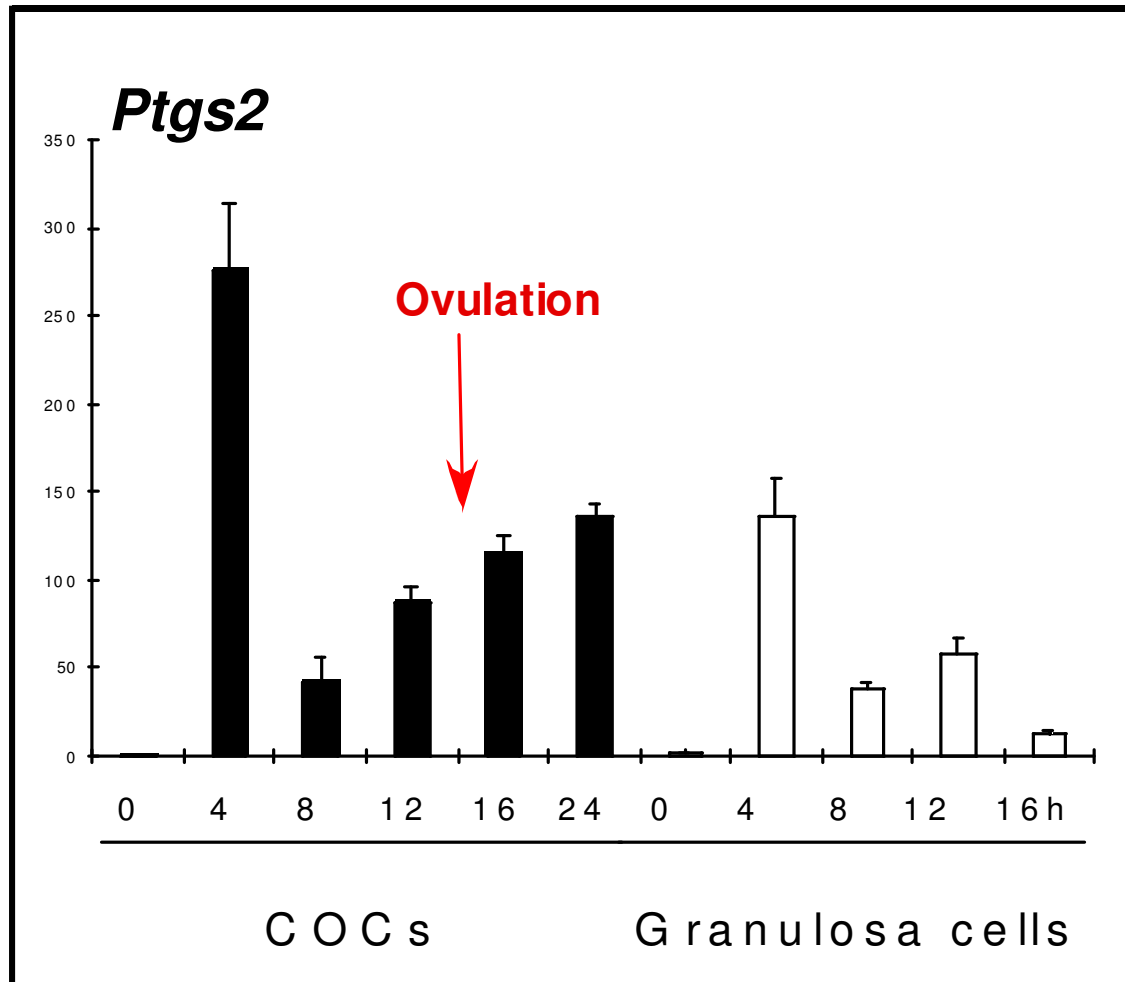
BCM
Baylor College of Medicine

**Ovulation is an inflammatory-like reaction
because levels of prostaglandins are high.
Espey 1980**



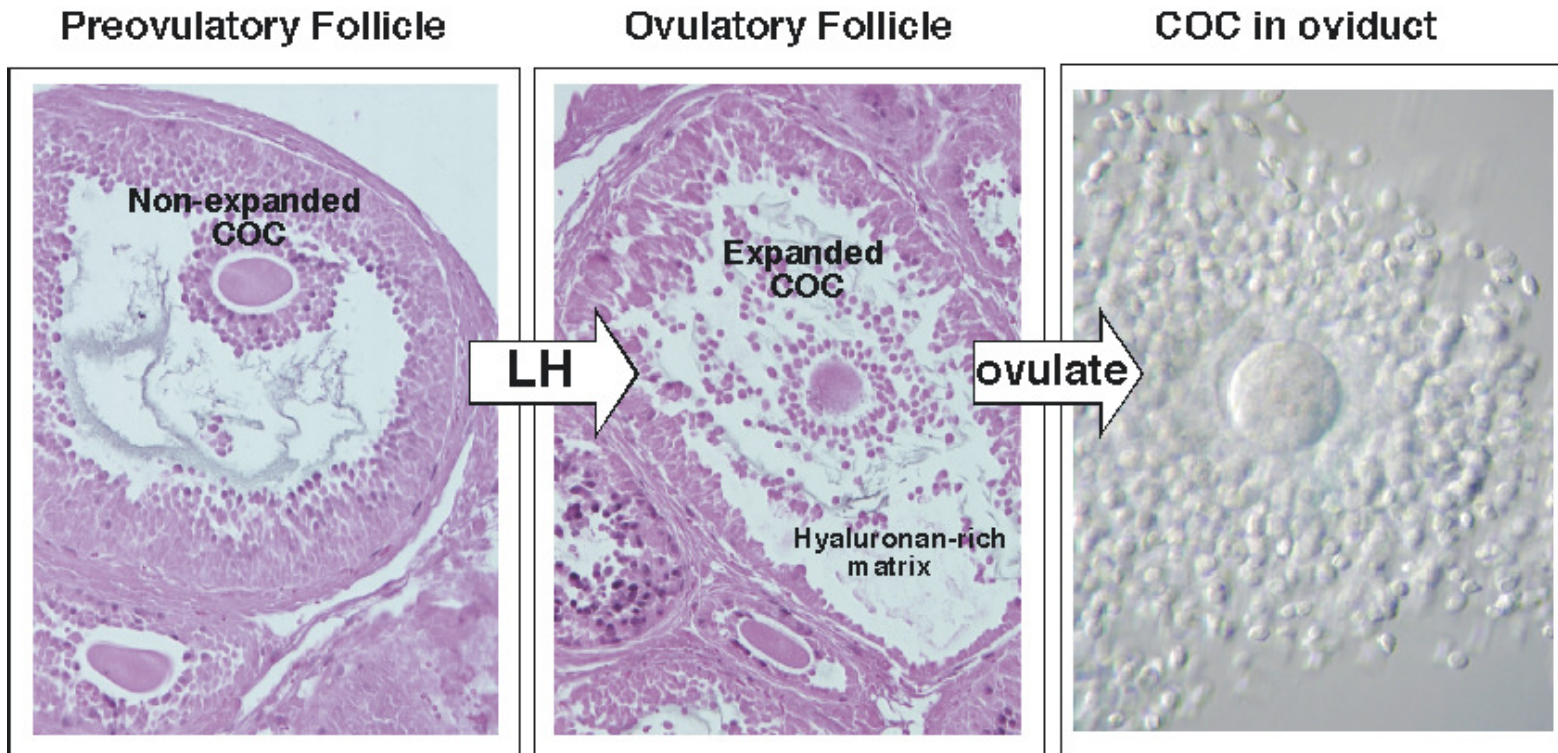
**Cumulus oocyte
complex
Follicle Rupture**

LH induction of prostaglandin synthase 2 (*Ptgs2*/COX2) is essential for ovulation; KO mice are infertile



Wong and Richards, *Mol Endocrinol*, 1991; Sirois et al *J Biol Chem*, 1992

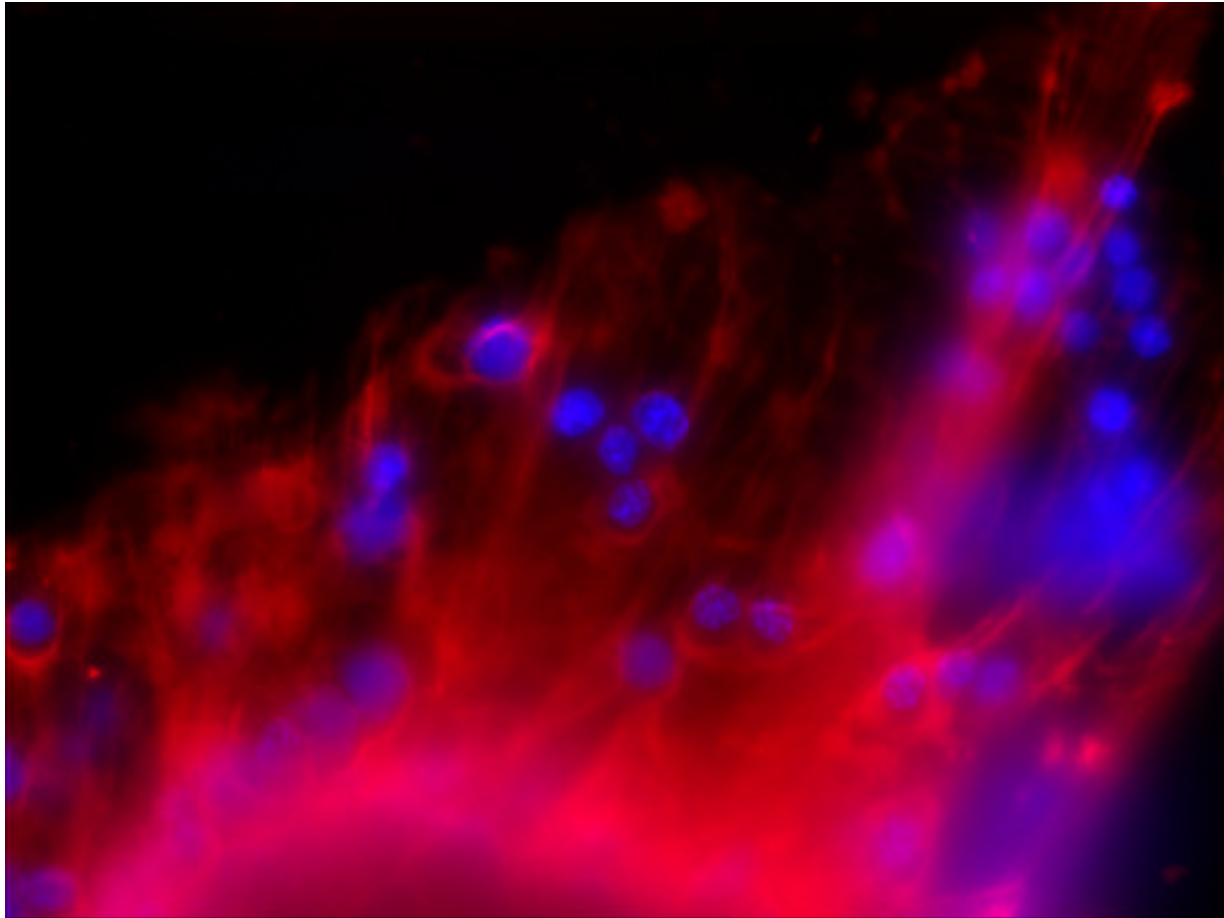
Cumulus cell-oocyte complex expansion is a highly specialized inflammatory-related process that is obligatory for successful ovulation



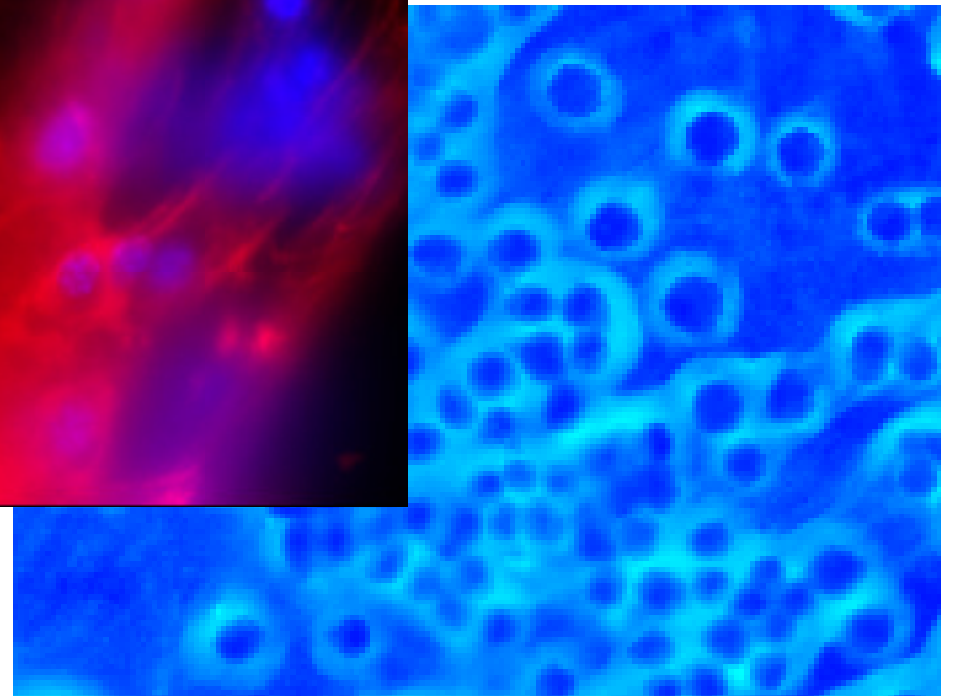
Release of a fertilizable oocyte within the cumulus oocyte complex (COC) requires:

- 1) The production and stabilization of an extracellular matrix
- 2) Genetic reprogramming cumulus cells
- 3) Meiotic maturation of the oocyte

Hyaluronic acid formation

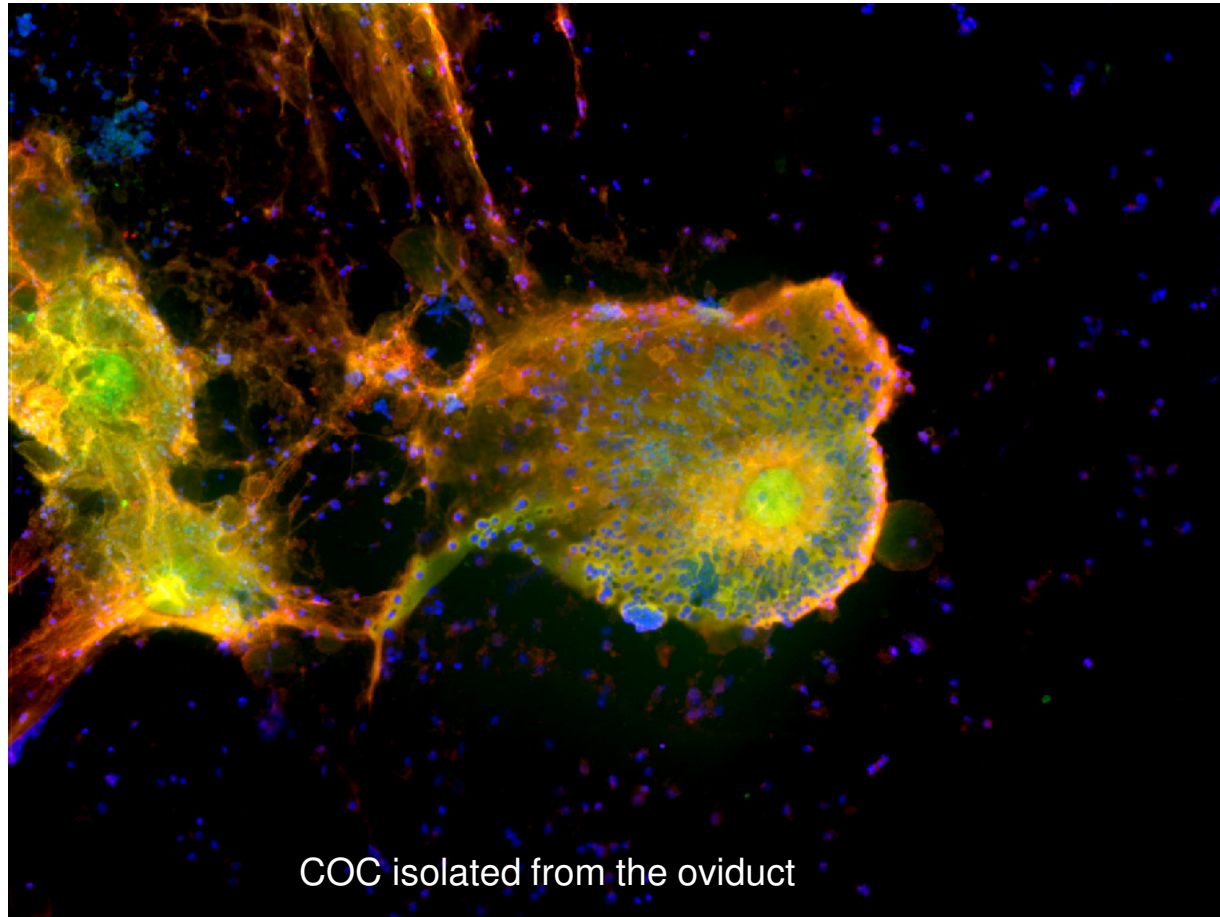


4h post hCG

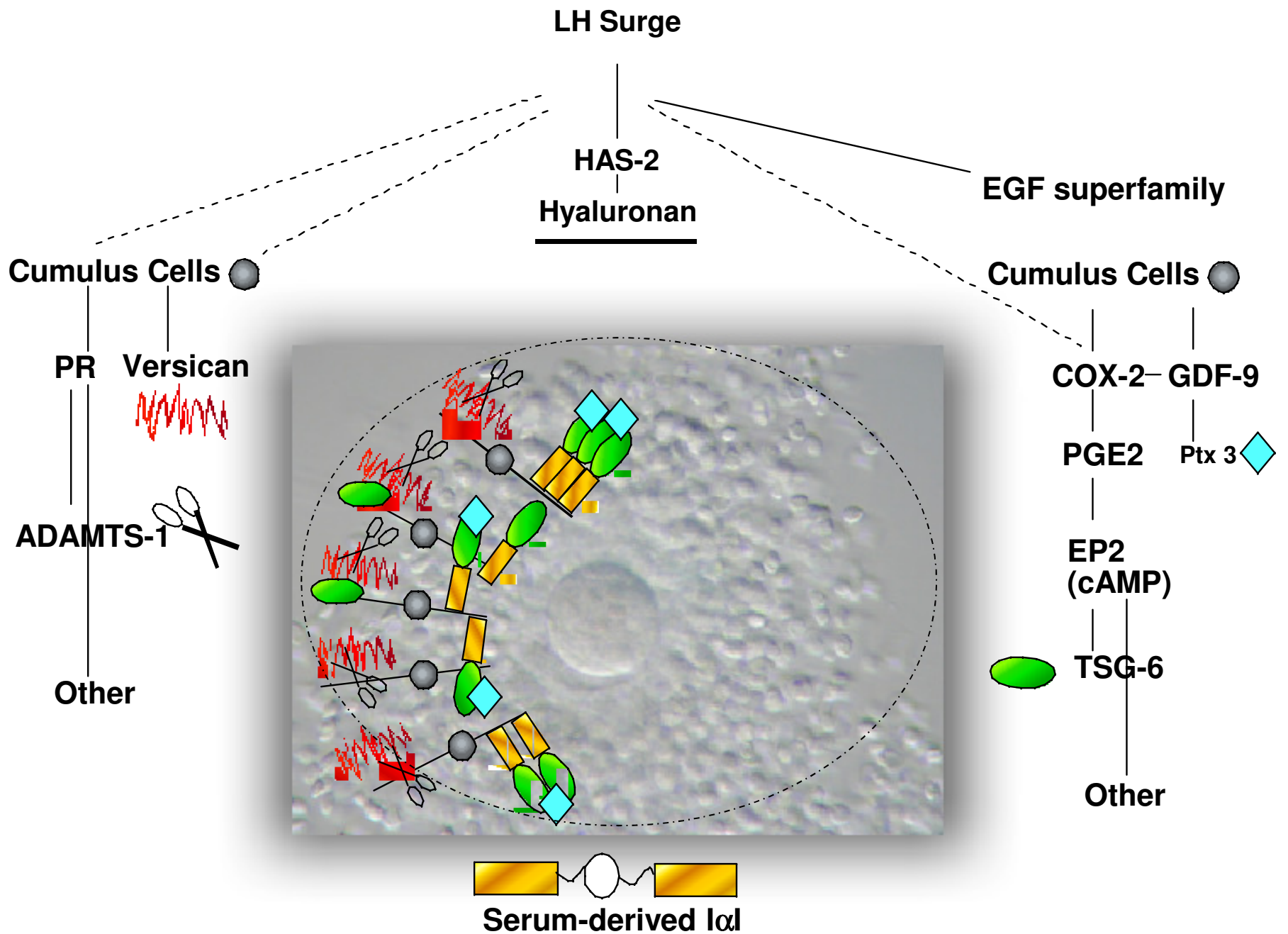


8h post hCG

HA binding proteins associated with inflammatory responses stabilize the matrix

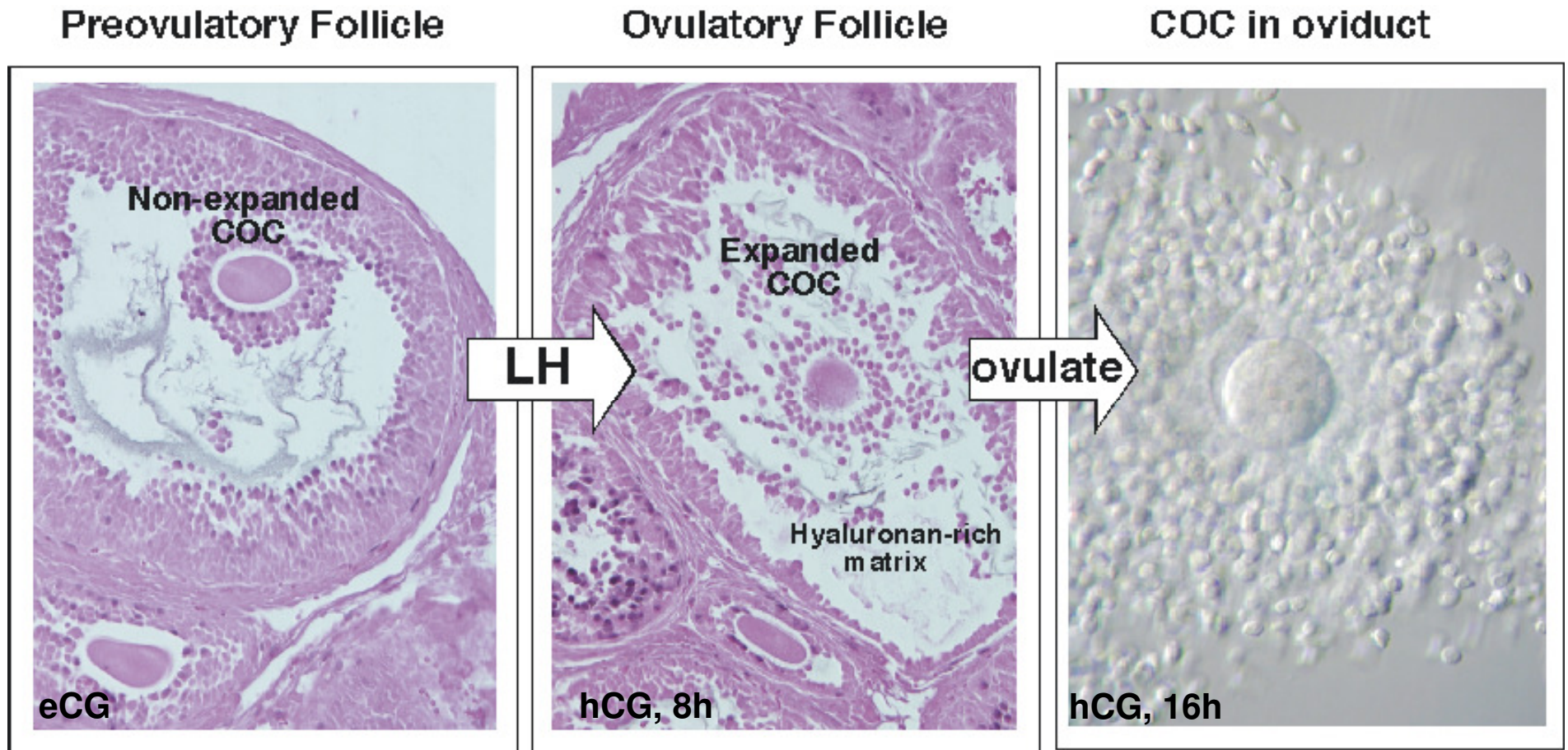


**HA, I α I and Dapi
(PTX3, TNFAIP6, versican)**



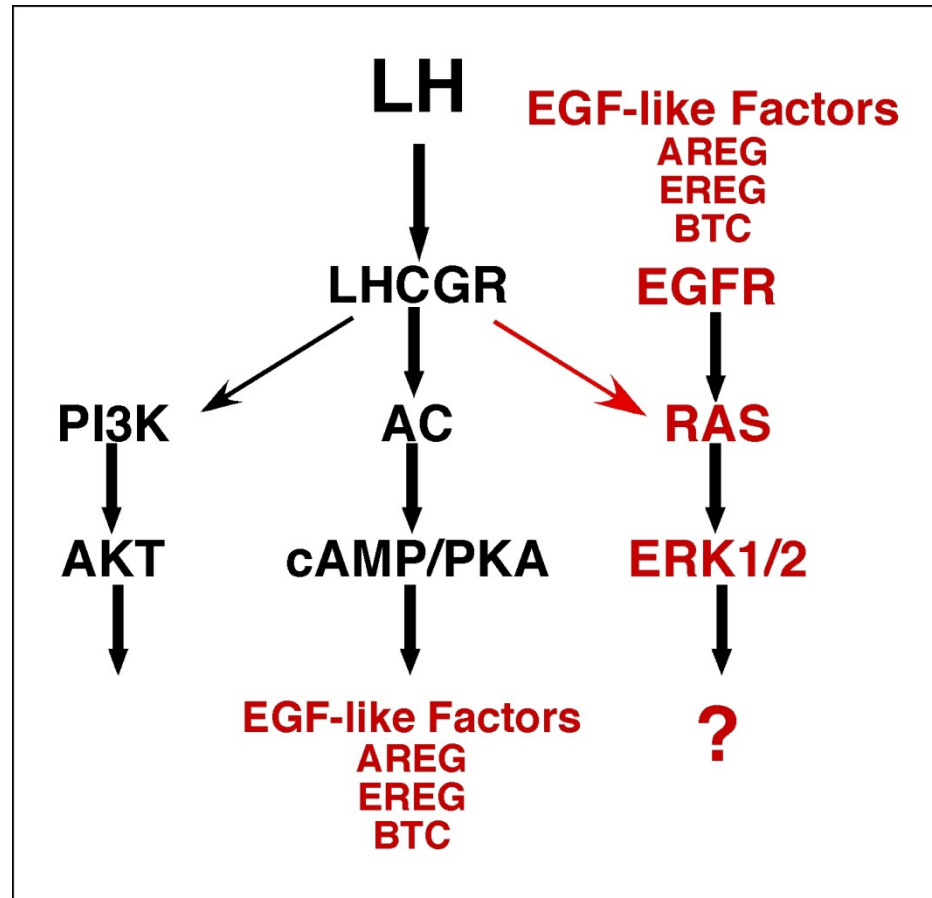
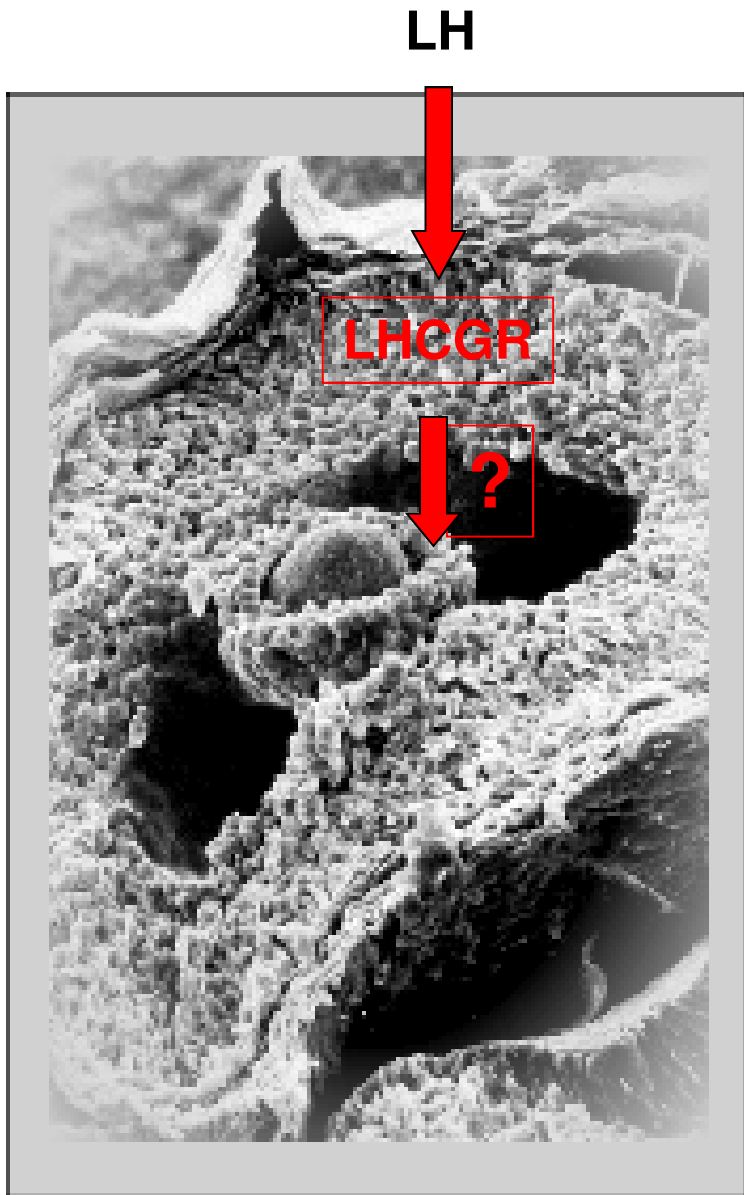
Mutant mouse models confirm that these genes are critical for COC expansion and fertility

How does LH induce COC expansion?



Because cumulus cells have a distinct cell fate, do cumulus cells express a unique set of genes?

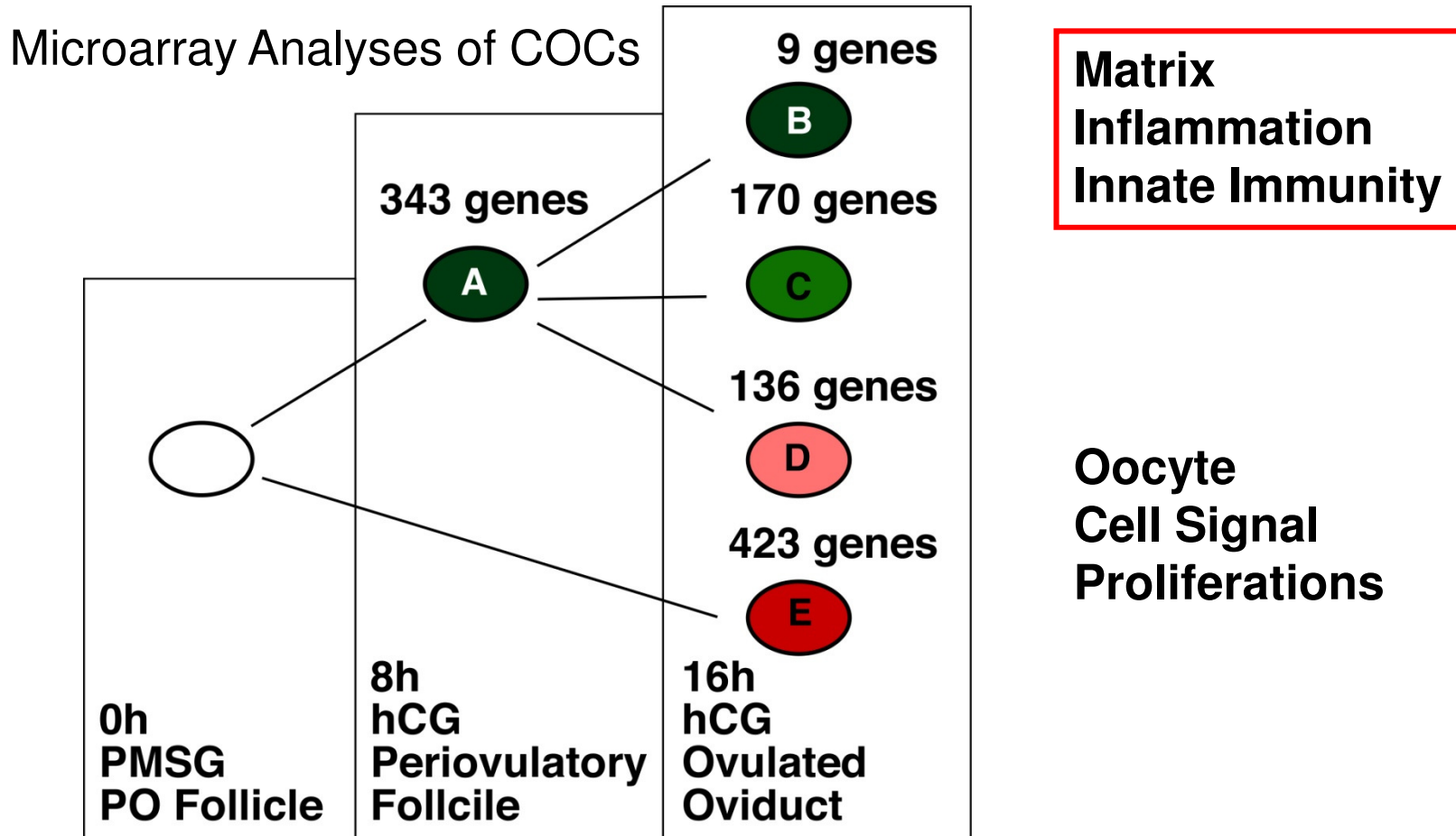
How does LH induce COC expansion?



AREG, like PGE and FSH, can induce COC expansion in culture.

QuickTime™ and a
Video decompressor
are needed to see this picture.

Do cumulus cells express a unique set of genes?



There are cell specific, as well as sequential and progressive, responses to LH

Rapid: EGF-like factors:AREG,EREG,BTC

PTGS2 and PTGER2/4

C/EBP beta

IL6

Intermediate:Toll receptor pathway

TLR2/4, MYD88

CD14

IL6

SNAP25

RUNX1/2

Late: Innate immune

PTX3

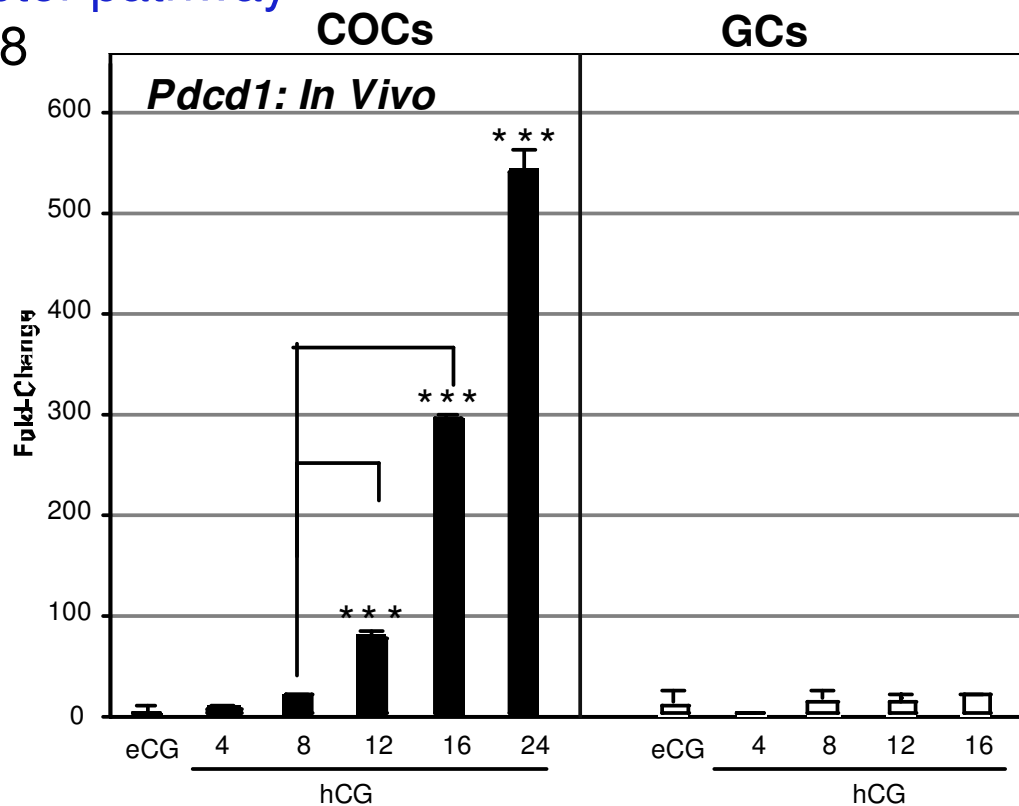
CD34

CD52

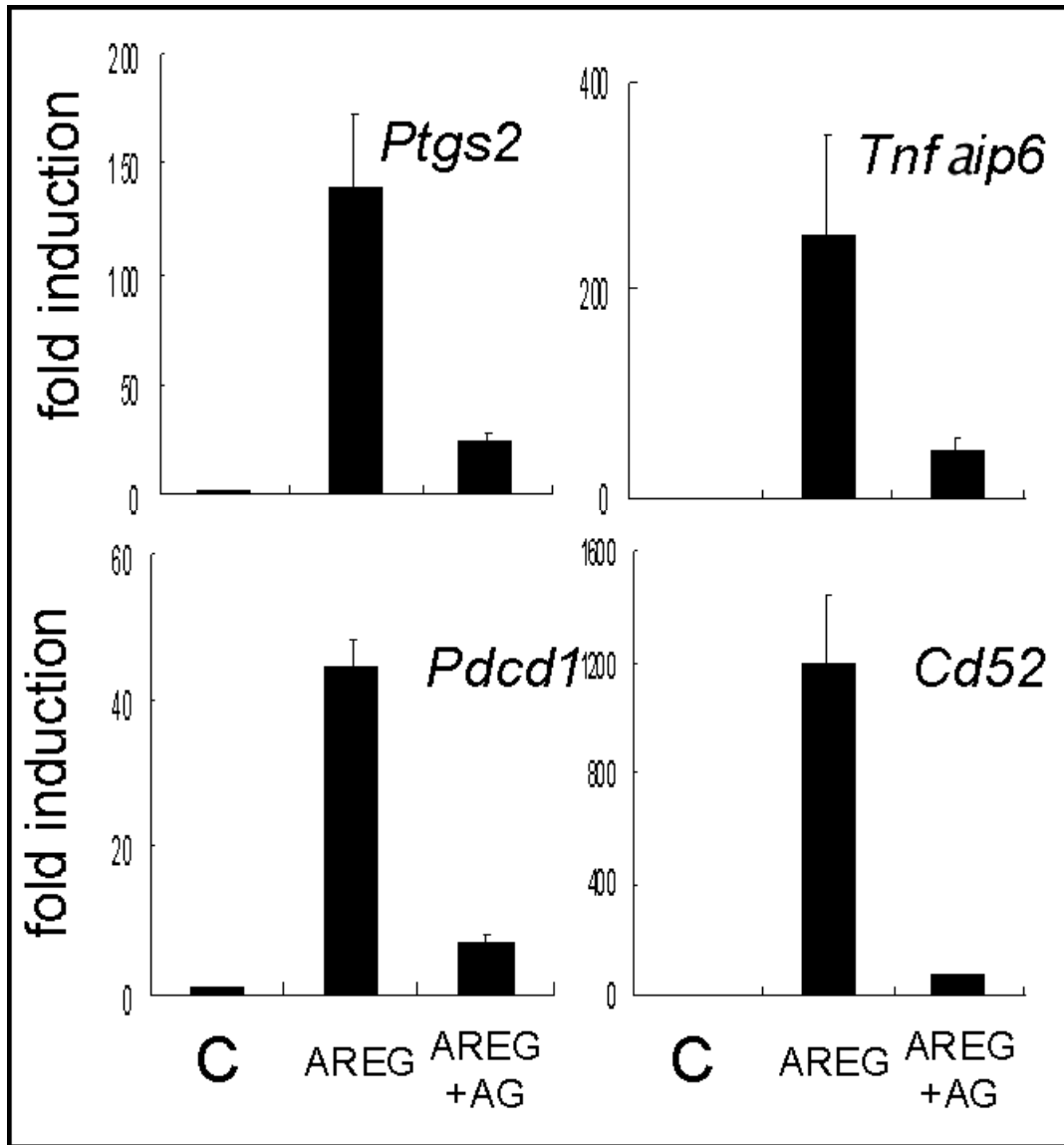
RUNX1/2

PDCD1

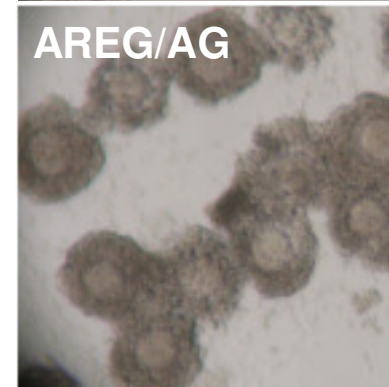
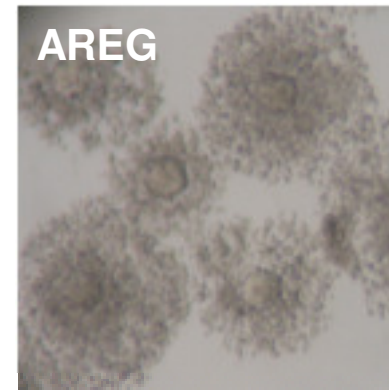
CD36



AREG induces genes in COCs in an EGF receptor dependent manner

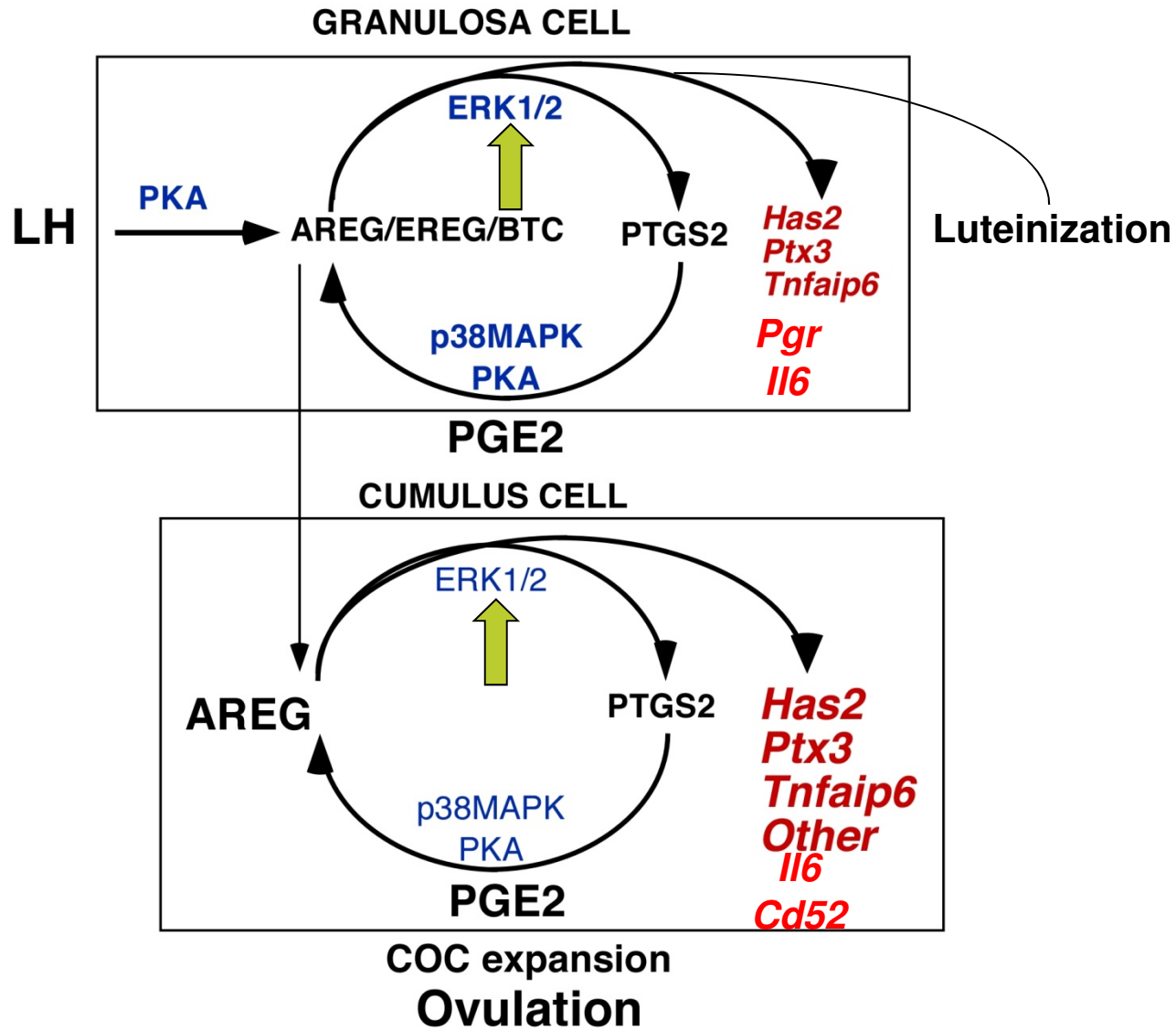


Matrix genes

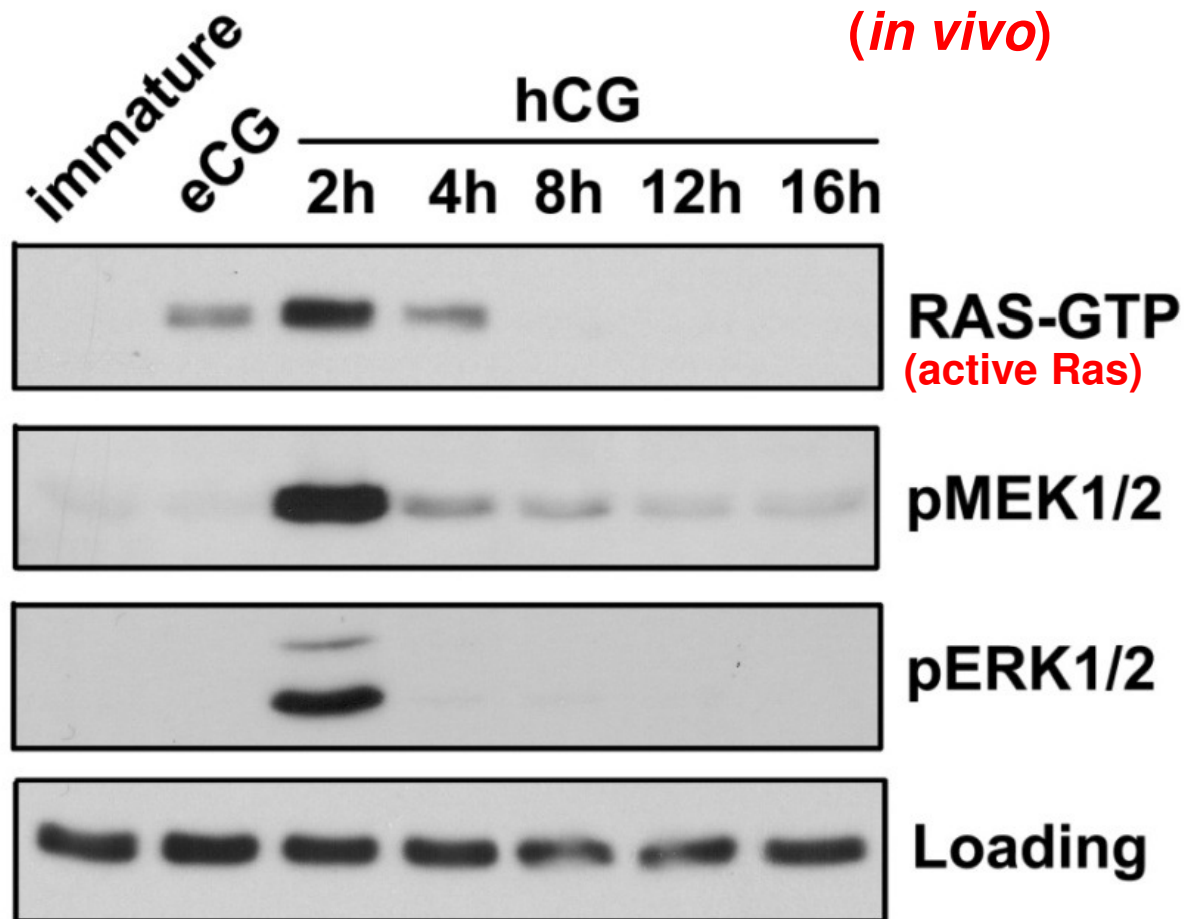


Innate immune-related genes

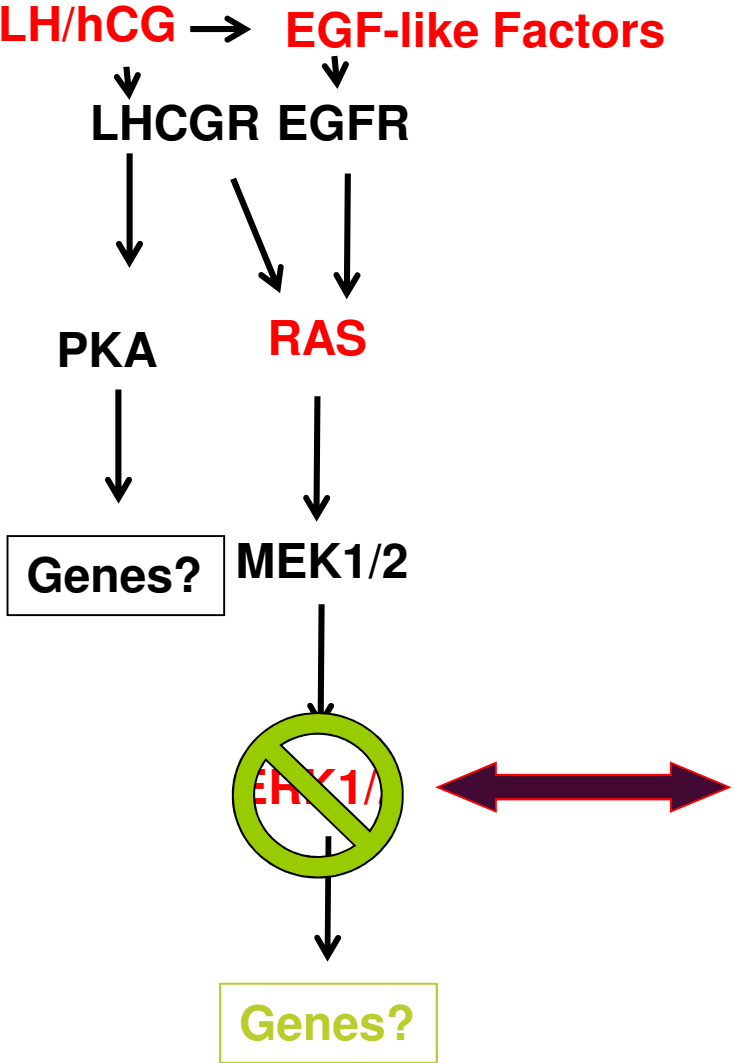
The AREG-PTGS2/PGE regulatory loop is essential for the induction of matrix related genes in granulosa cells and cumulus cells.



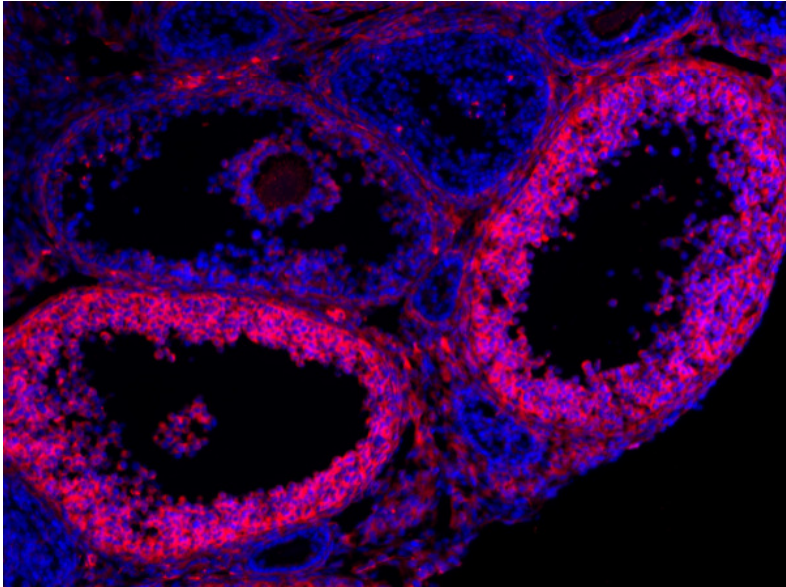
LH/hCG activation of **RAS** and **ERK1/2** in granulosa cells *in vivo* is rapid, transient and tightly coordinated



If LH activates both the PKA and RAS/ERK1/2 pathways, what genes that control ovulation, COC expansion and luteinization are downstream targets of each pathway?



hCG 2h



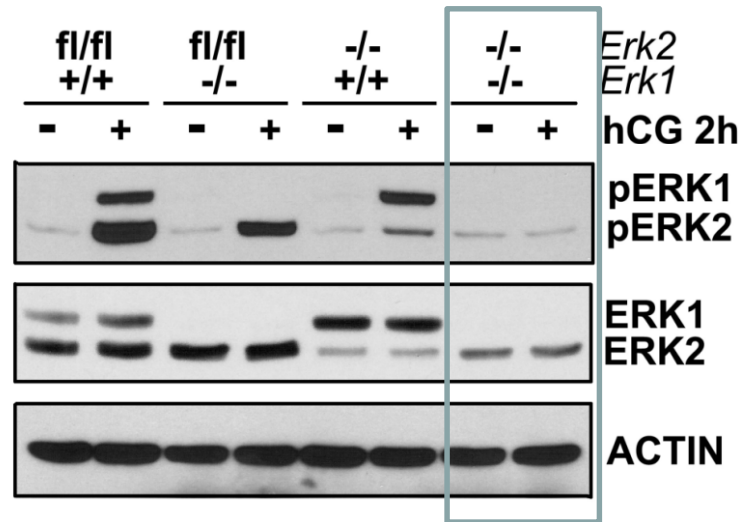
DAPI

pERK1/2

- *Erk1* knockout mice are viable and fertile (Pagès *et al*, Science 1999).
- *Erk2* null mice die at E6.5
- Therefore, *Erk2*^{fl/fl} mice have been generated (Fischer *et al*, Immunity 2005).

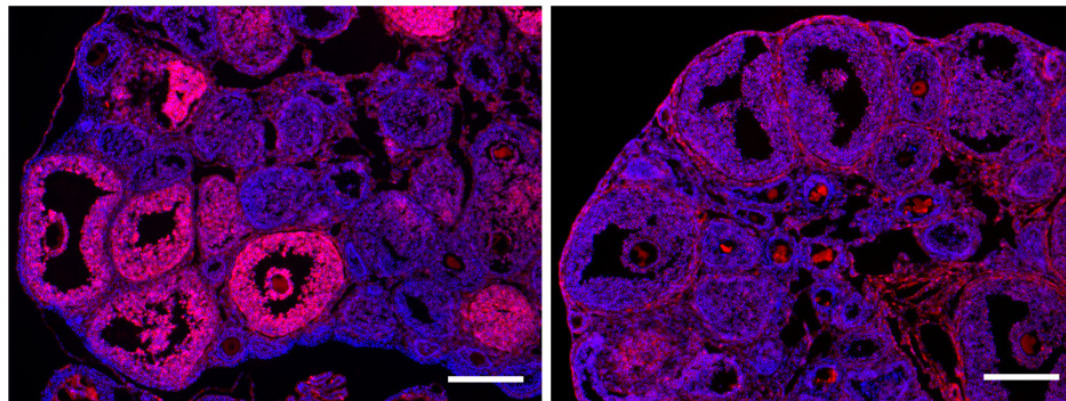
To generate a double KO mouse, *Erk2* was disrupted in granulosa cells of the *Erk1* null strain by mating *Erk1*^{-/-} mice with *Erk2*^{fl/fl}; *Cyp19-Cre* mutant mice.

ERK1/2 were successfully deleted in granulosa cells



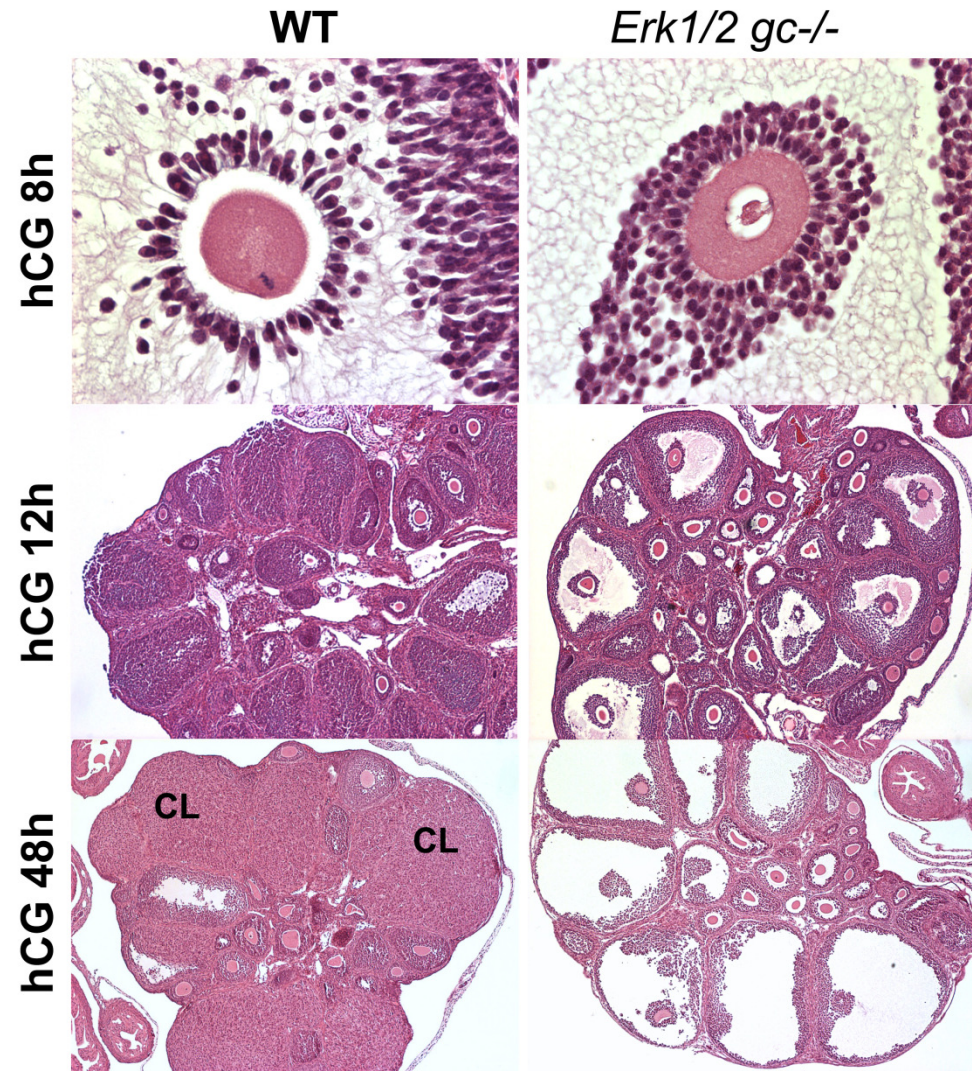
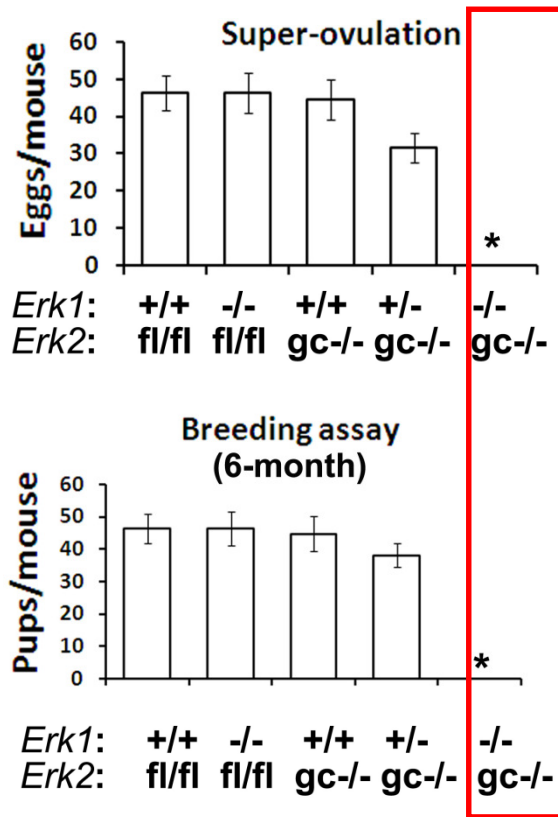
WT

Erk1/2 gc-/-

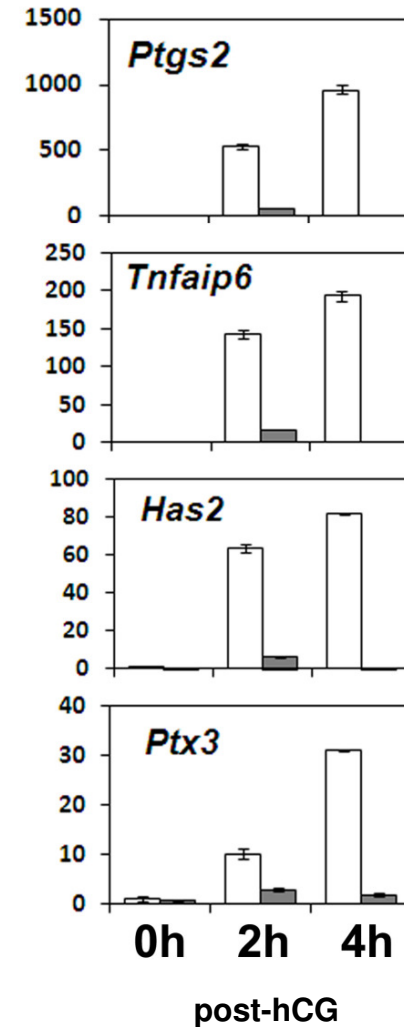
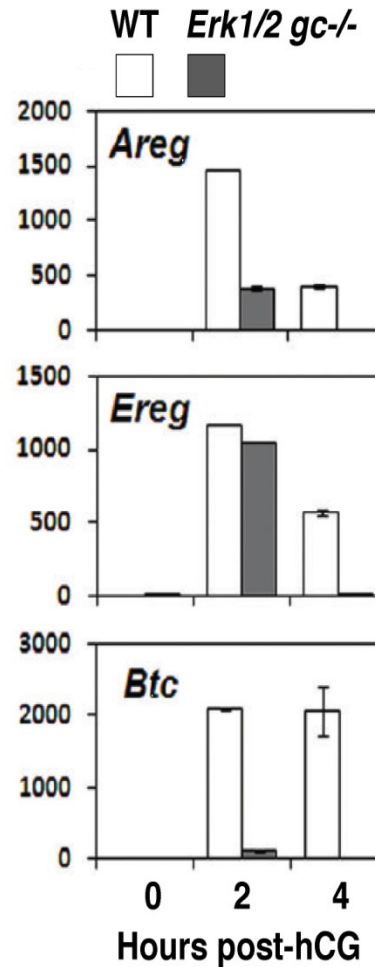
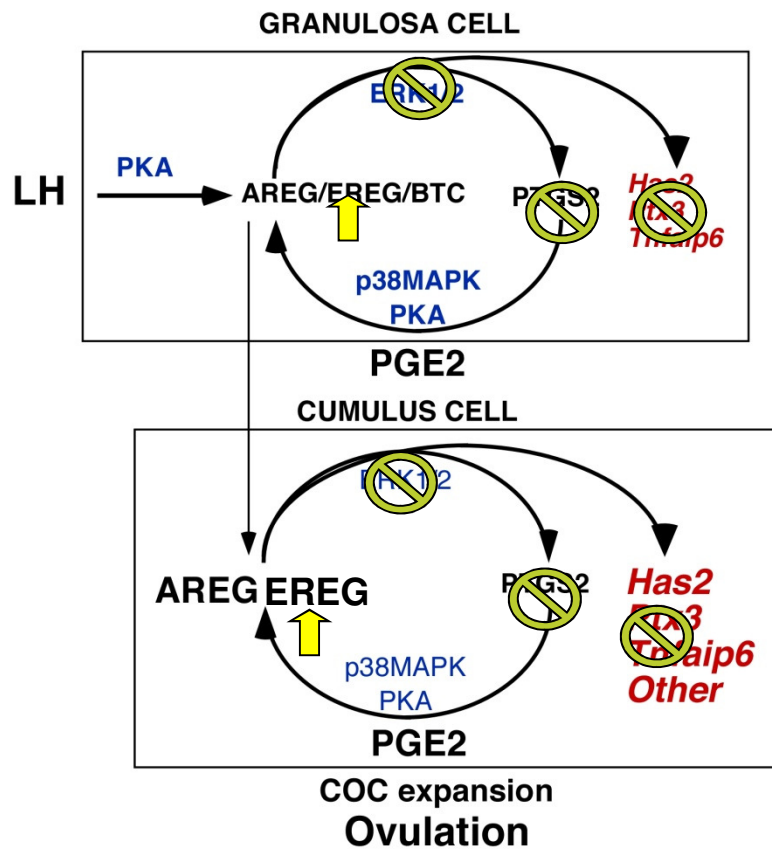


DAPI/pERK1/2

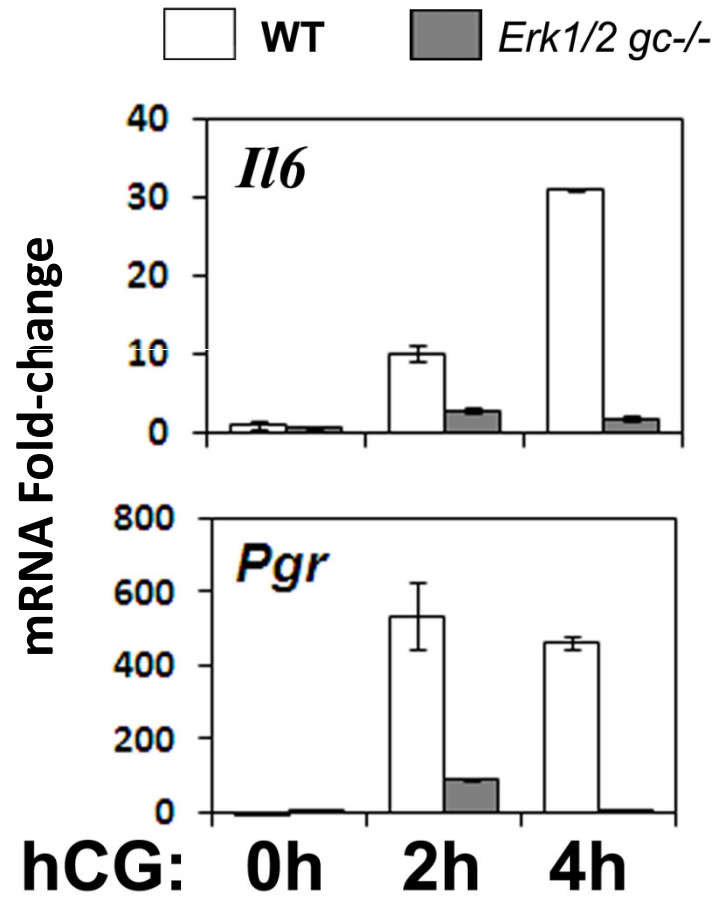
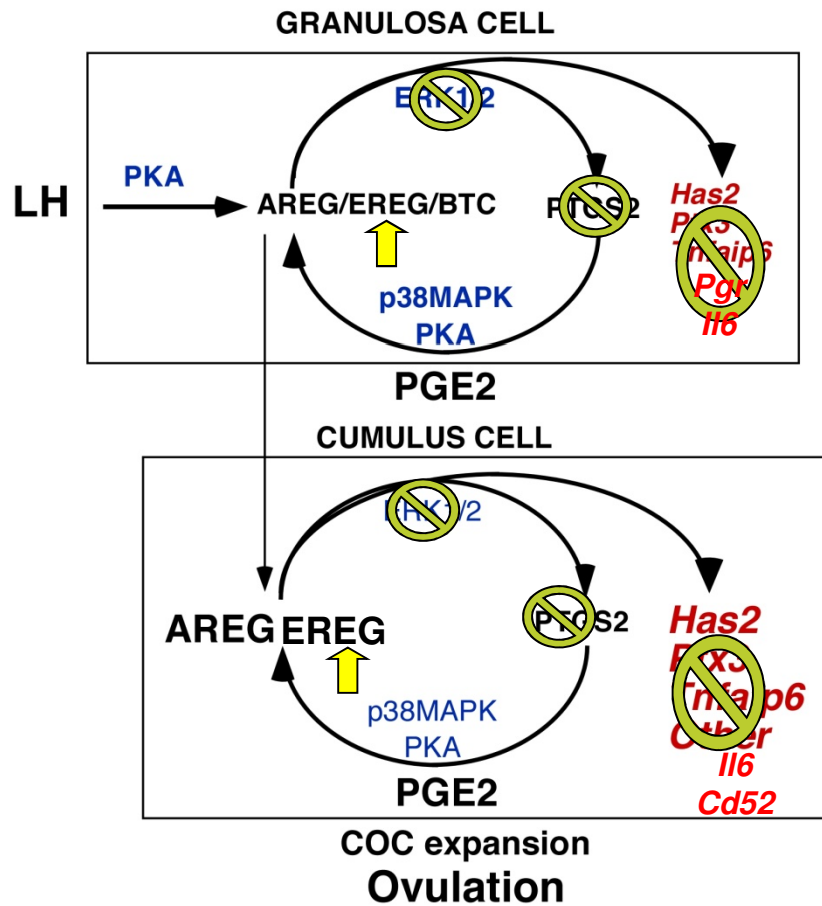
Ovulation, oocyte maturation, cumulus expansion and luteinization are blocked in *Erk1/2^{gc/-}* mice



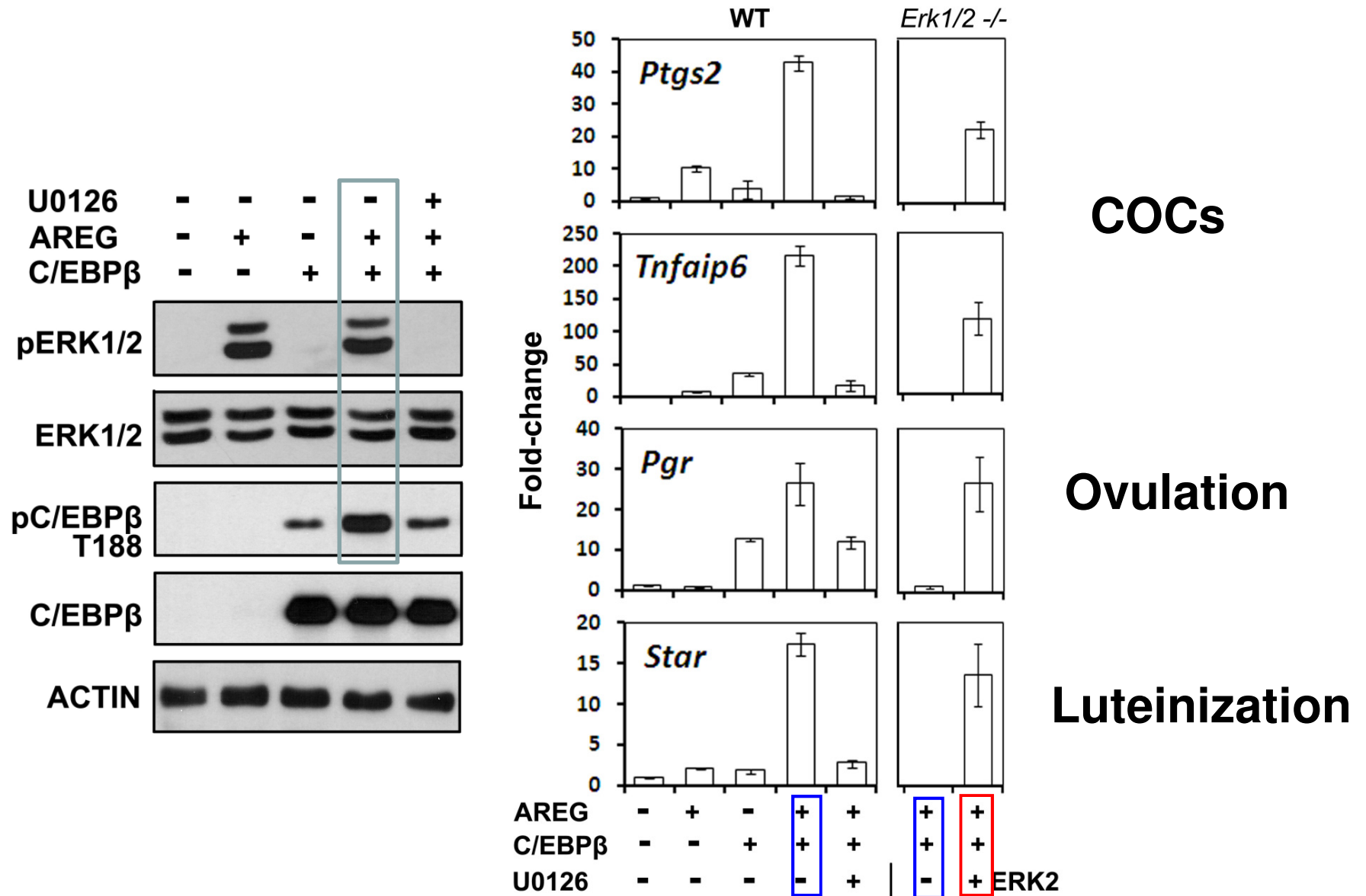
ERK1/2 globally regulate the expression of LH-target genes



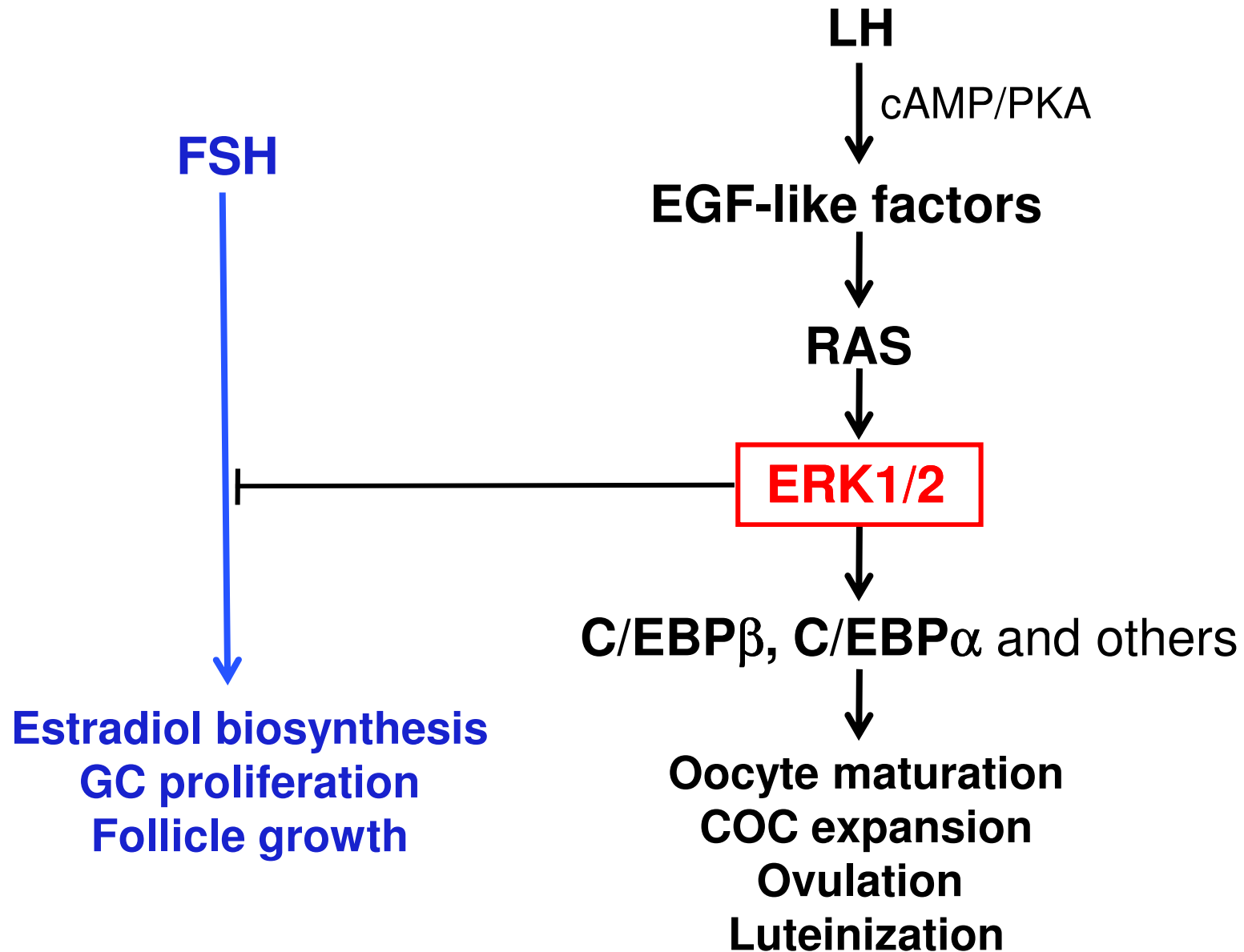
LH/hCG-induced synthesis of *Il6* and *Pgr* mRNA is abolished in ERK1/2-depleted follicles



ERK1/2 induce the expression of selected LH-target genes by activating C/EBP β



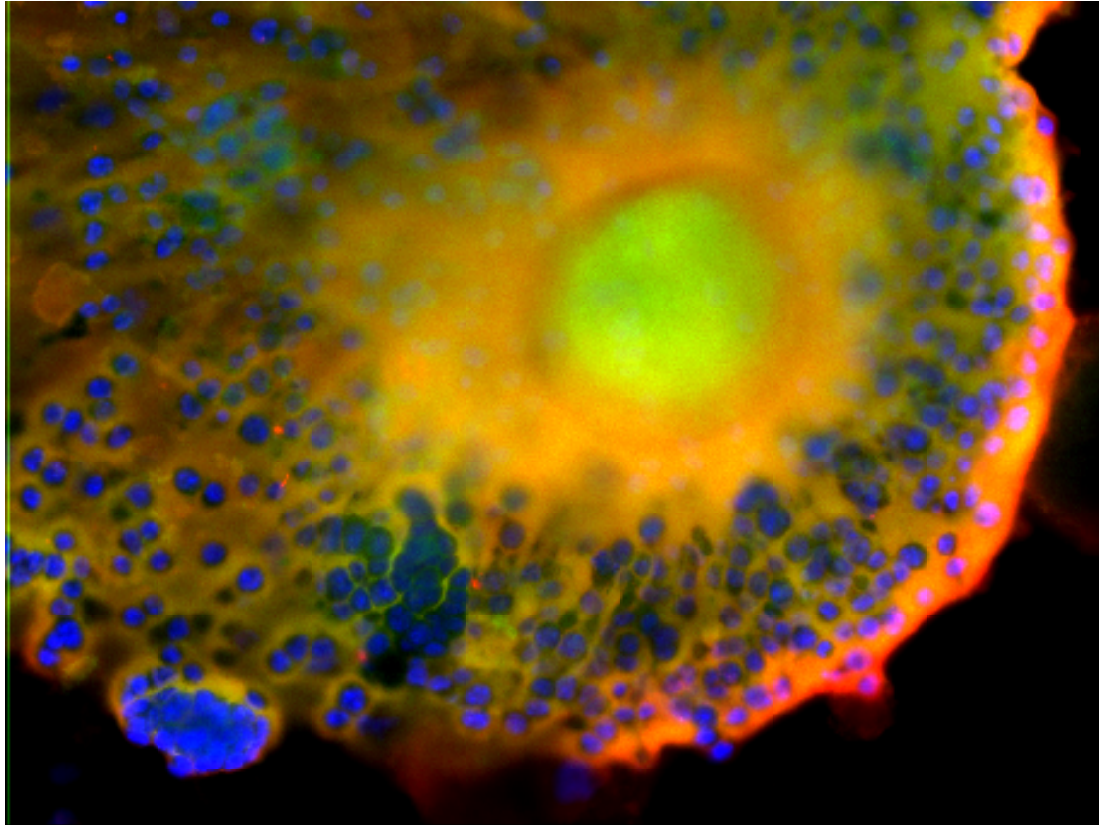
ERK1/2 control the molecular switch by which LH reprograms granulosa cells and cumulus cells in preovulatory follicles.



What other genes and signaling pathways are downstream of the ERK1/2 molecular switch?

Genes controlling:

Inflammatory responses
Innate immune processes
Terminal differentiation and
Cell cycle arrest



Is the COC matrix a protective shield?
Does the matrix exert specific functions?

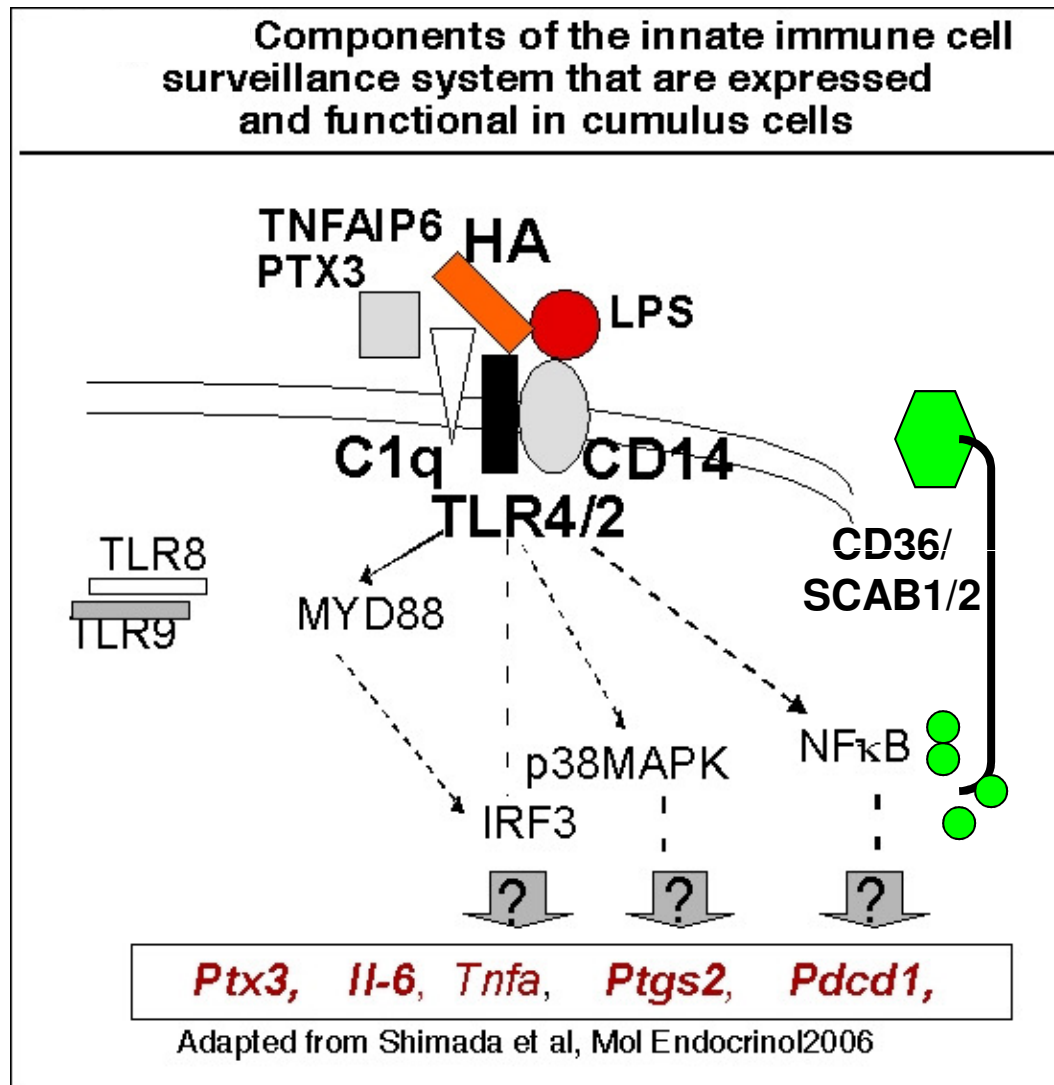
Do cumulus cells control functions beyond the matrix?

The immune system is a surveillance system that **recognizes** “self” from “non-self” or “altered-self” via pathogen recognition receptors (PRRs): CD14, Toll-like receptors (**TLRs**), C1q leading to transcription of ***Il6***, ***Tnfα*** and ***Ptgs2***.

Macrophages **remove** “non-self” (bacteria;LPS) or “altered self” (apoptotic cells) via scavenger receptors (**CD36**, **SCARB1/II**) that are induced by cytokines, such as **IL6**.

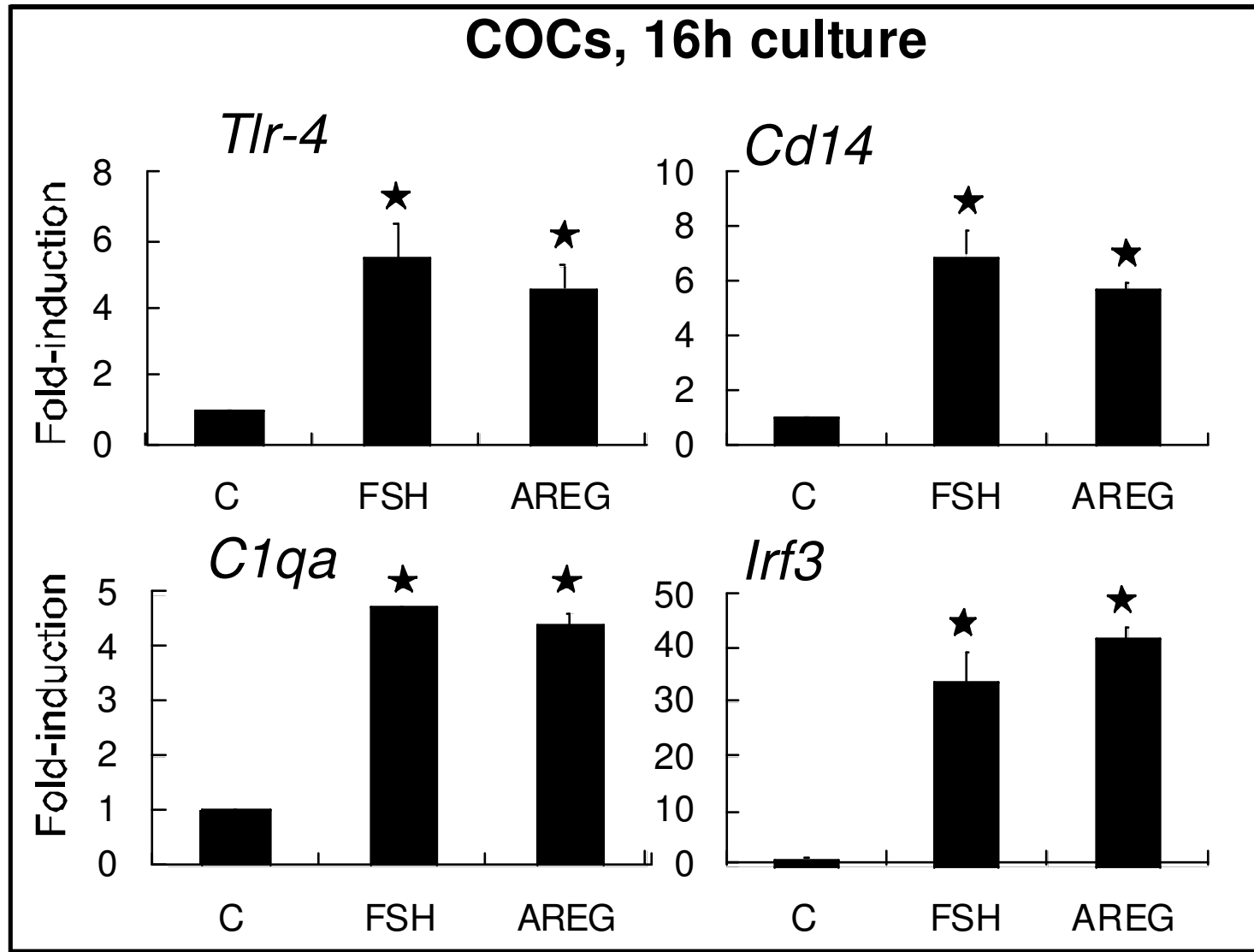
If cumulus cells have immune-related functions, do they express genes related to surveillance functions?

Model of matrix and immune cell related factors

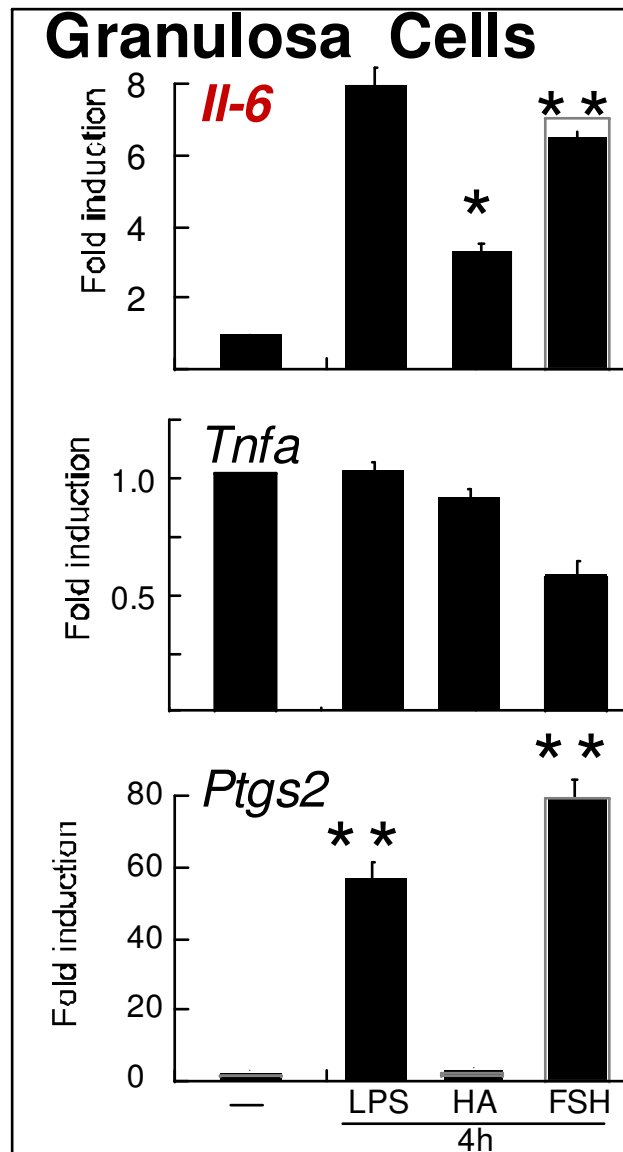
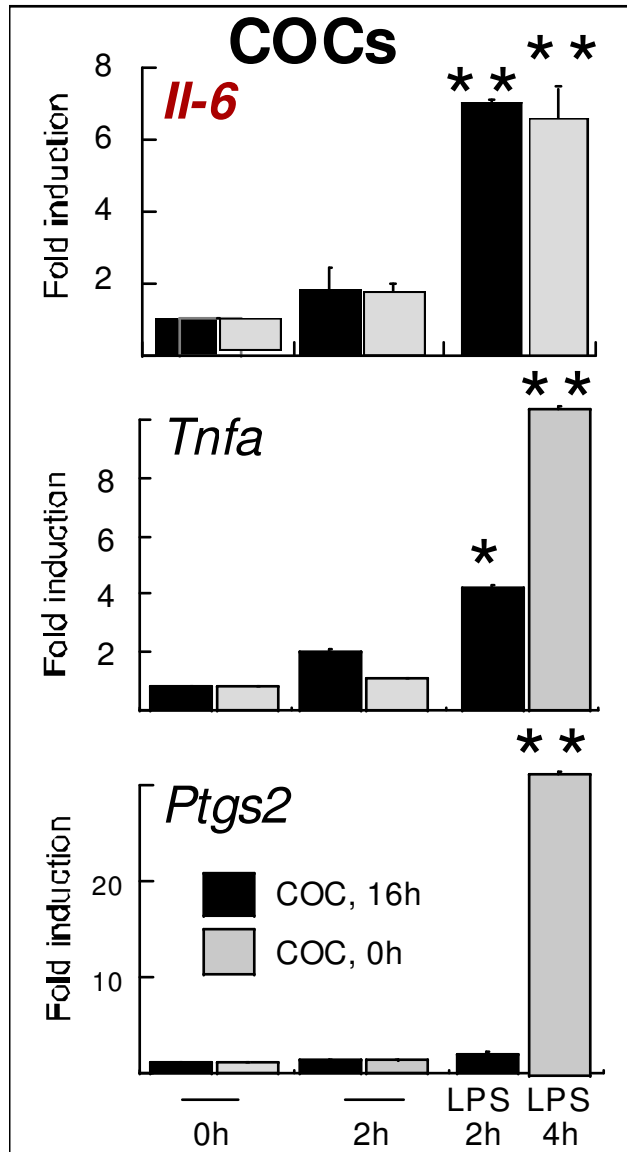


Functions *in vivo*: Matrix molecules **HA**, surveillance (sperm), apoptosis (cumulus cells)

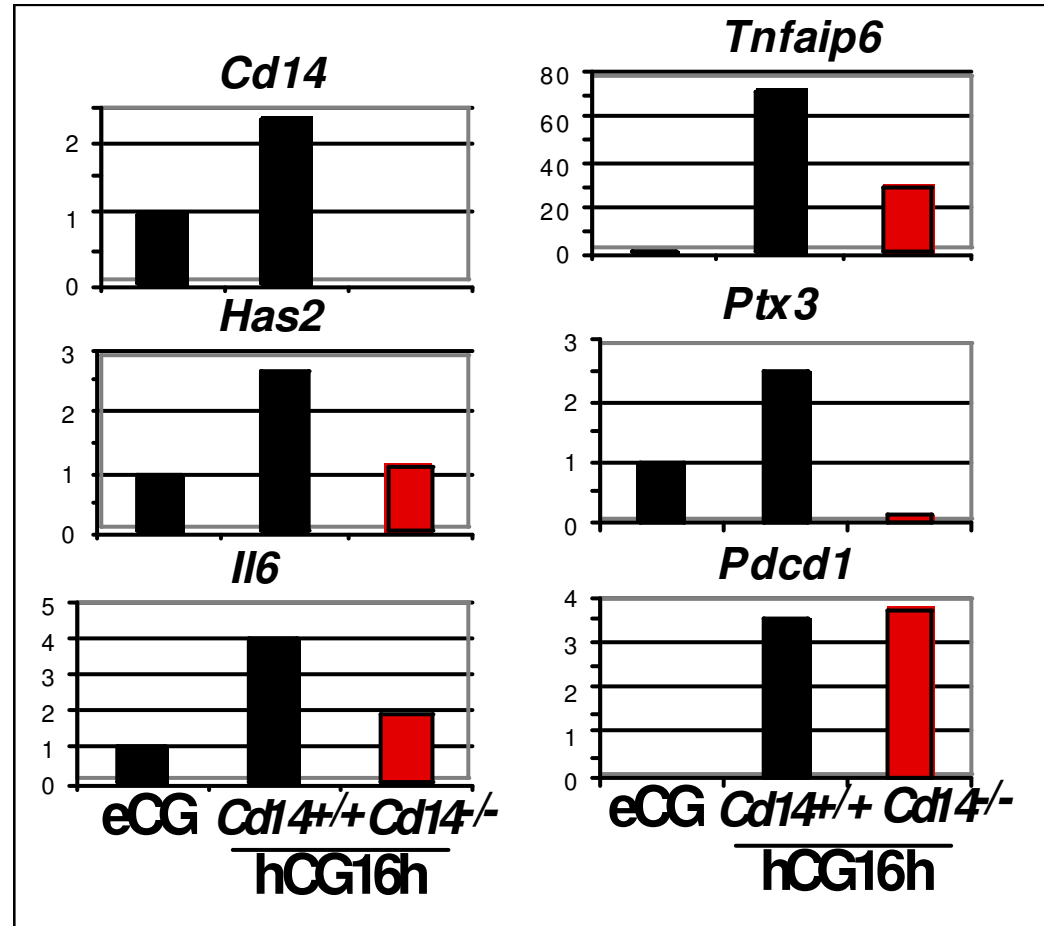
Components of the TLR receptor pathway are induced by FSH and AREG in cultured mouse COCs



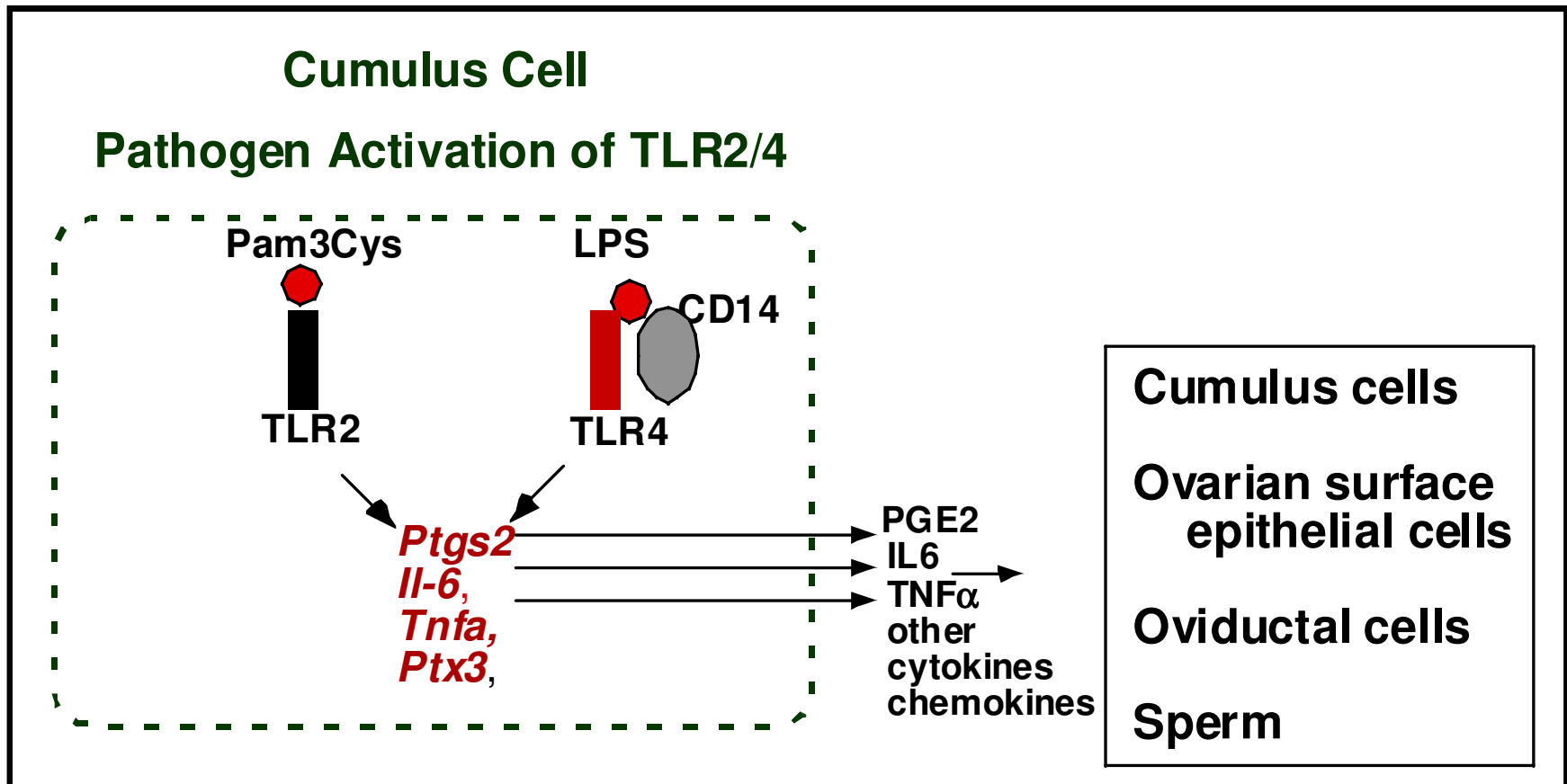
TLRs present in COCs are functional.
 LPS induces expression of *Il6*, *Tnfa* and *Ptgs2* mRNAs



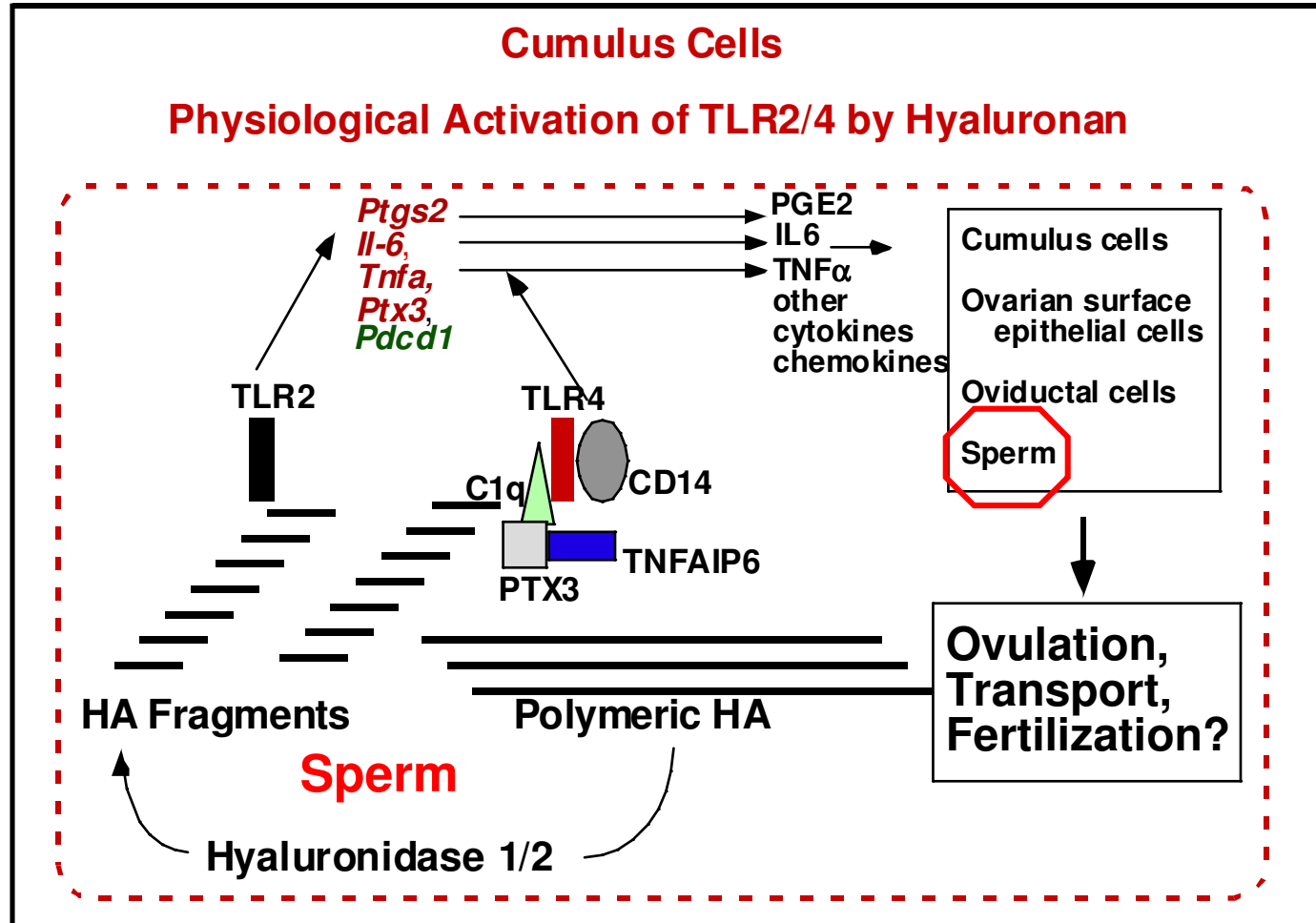
Matrix genes are mis-regulated in COC isolated from *Cd14* null mice



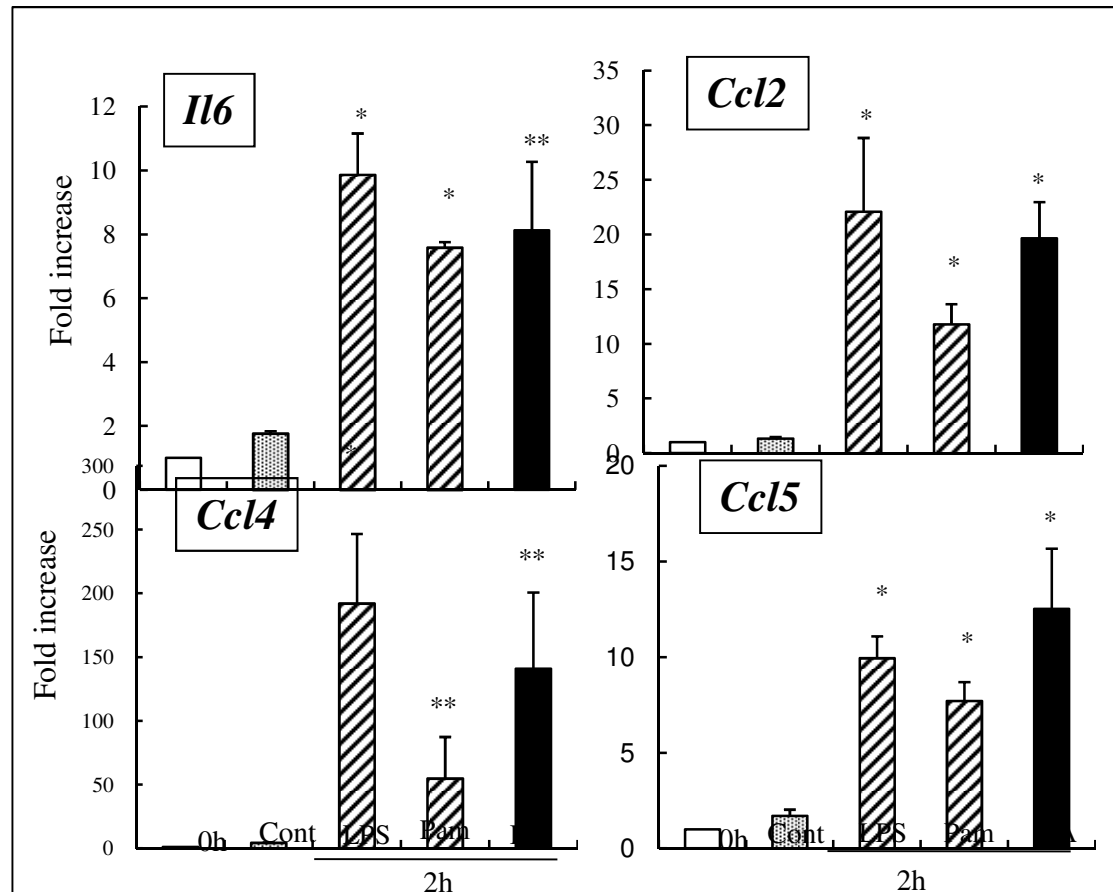
Cumulus cells can release potent cytokines
in response to **pathogens**



Cumulus cells can release potent cytokines in response to physiological stimuli such as HA fragments



Other cytokines and chemokines are induced in COCs (and granulosa cells) in response to pathogens and HA fragments

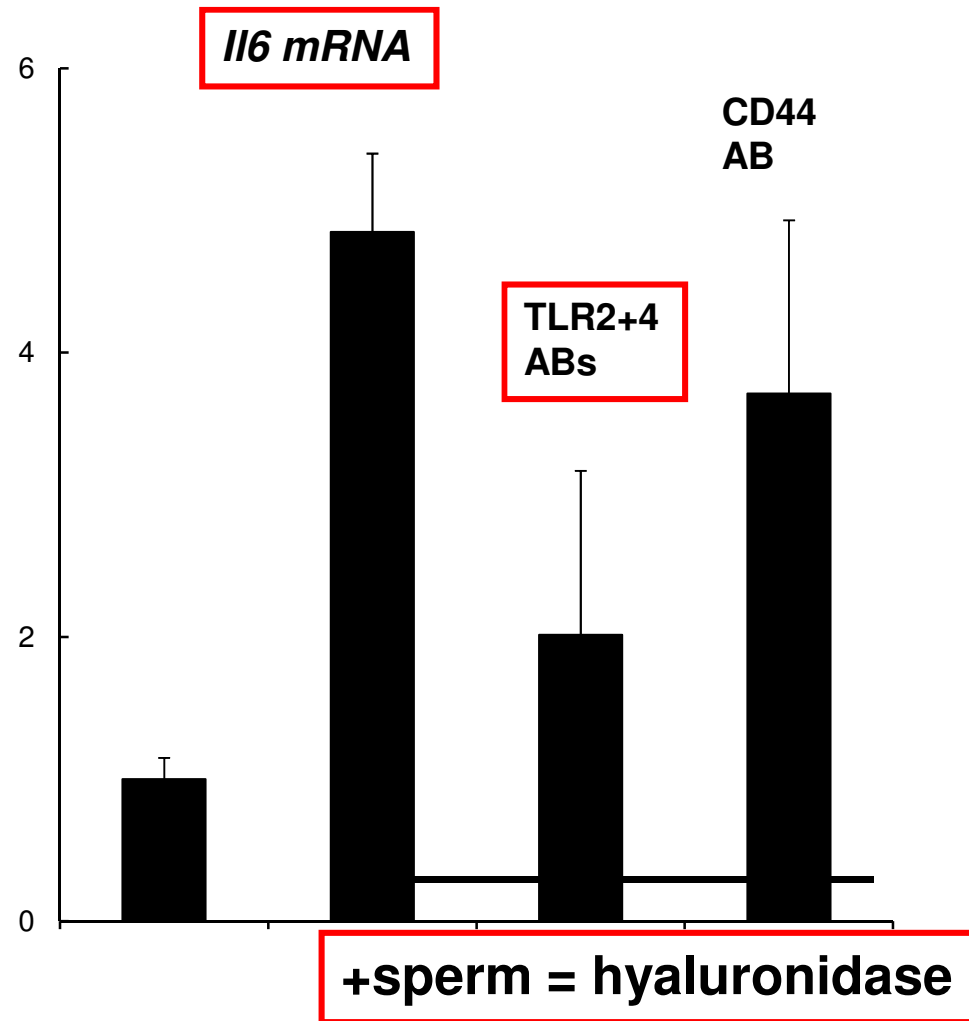


Ccl2/MCP1: monocyte chemotactic protein 1

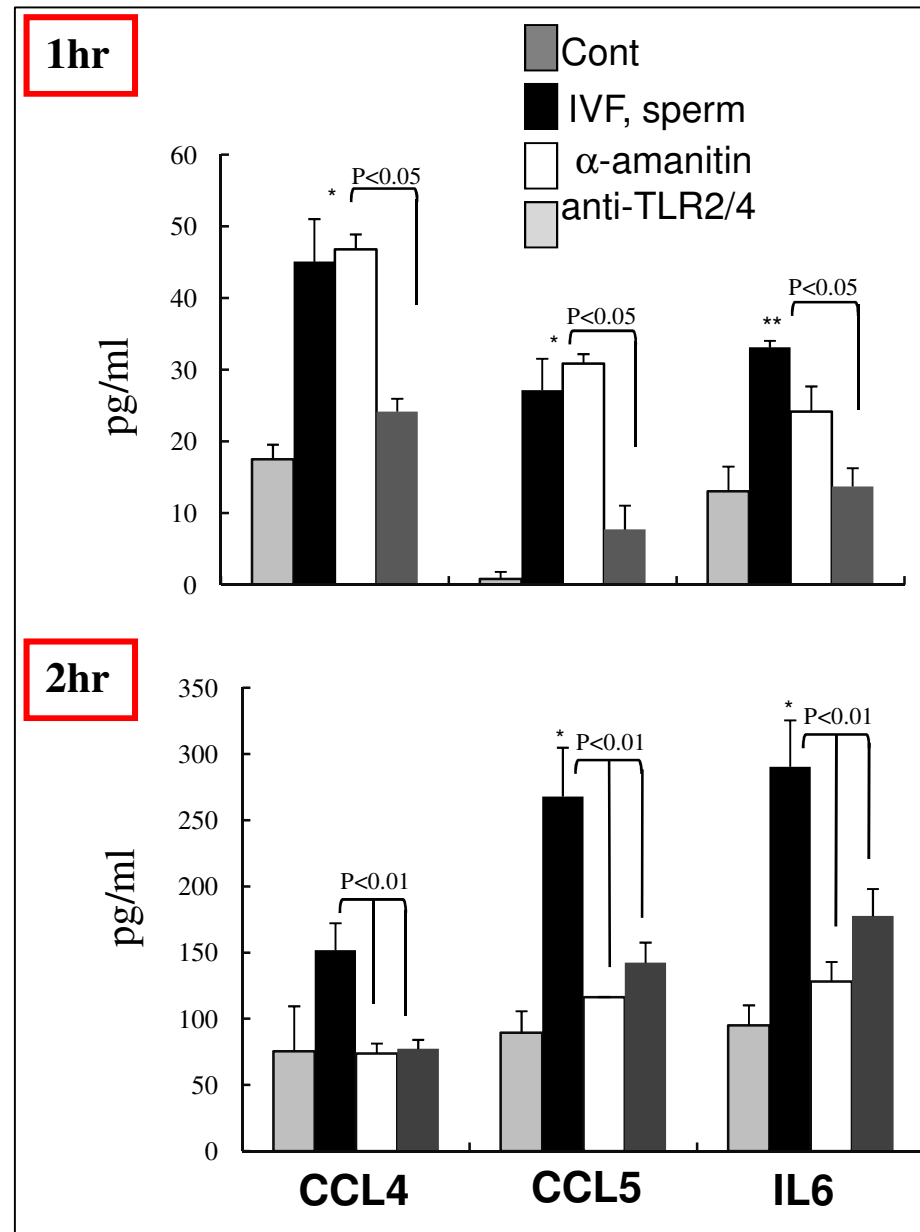
Ccl4/MIP1 β : macrophage inflammatory protein 1 β

Ccl5/ RANTES: regulated upon activation, T-cell expressed and secreted

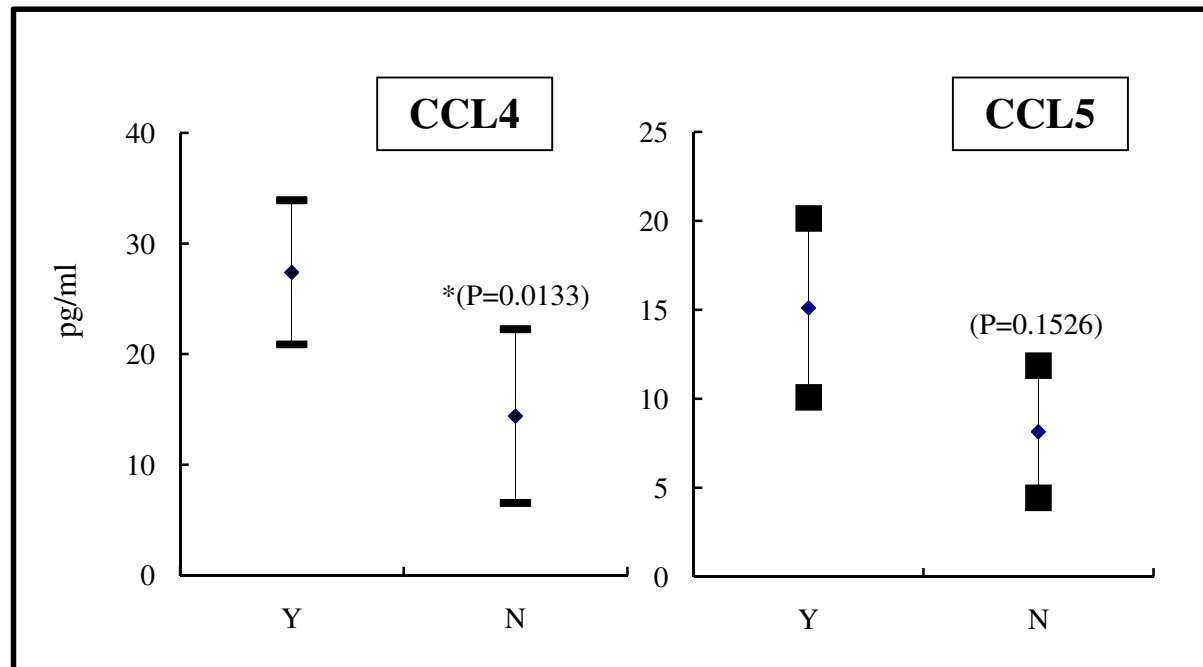
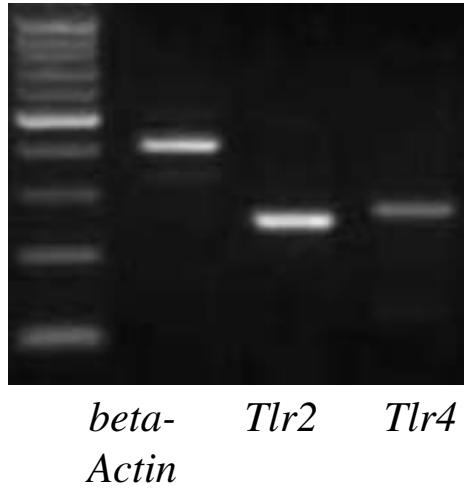
Sperm activate the TLR2/4 pathways in COCs and induce expression of *Il-6*.



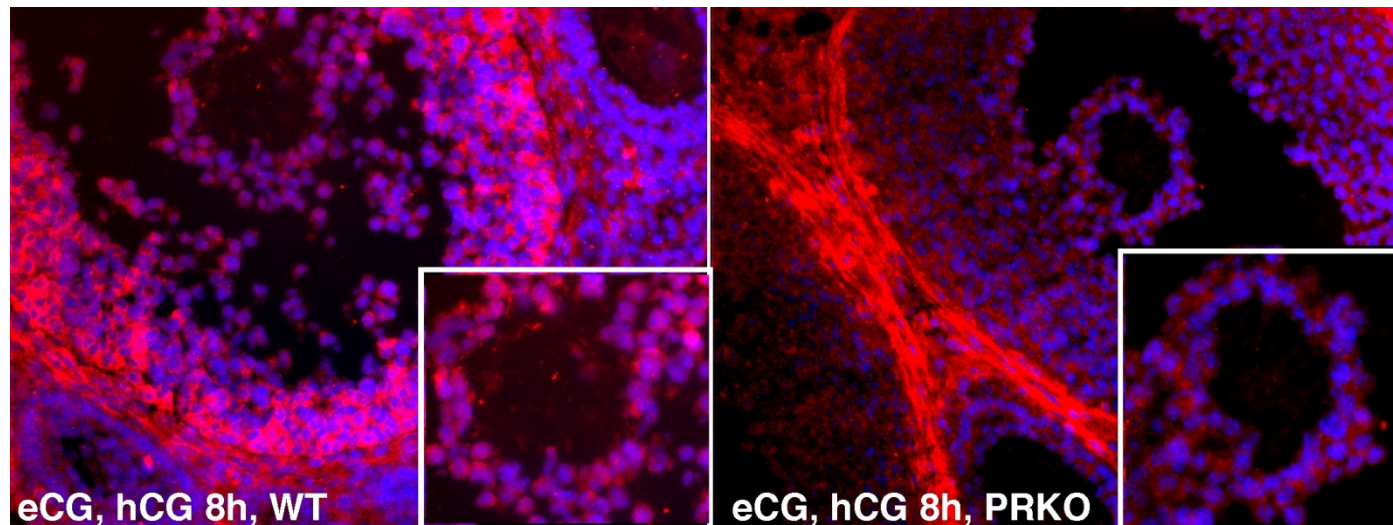
Cytokines and chemokines are rapidly released and produced by cumulus cells during IVF procedures in a TLR2/4-dependent mechanism.



Human COCs express *Tlr2* and *Tlr4* mRNAs and levels of chemokines released
In IVF protocols are related to fertility success in women.

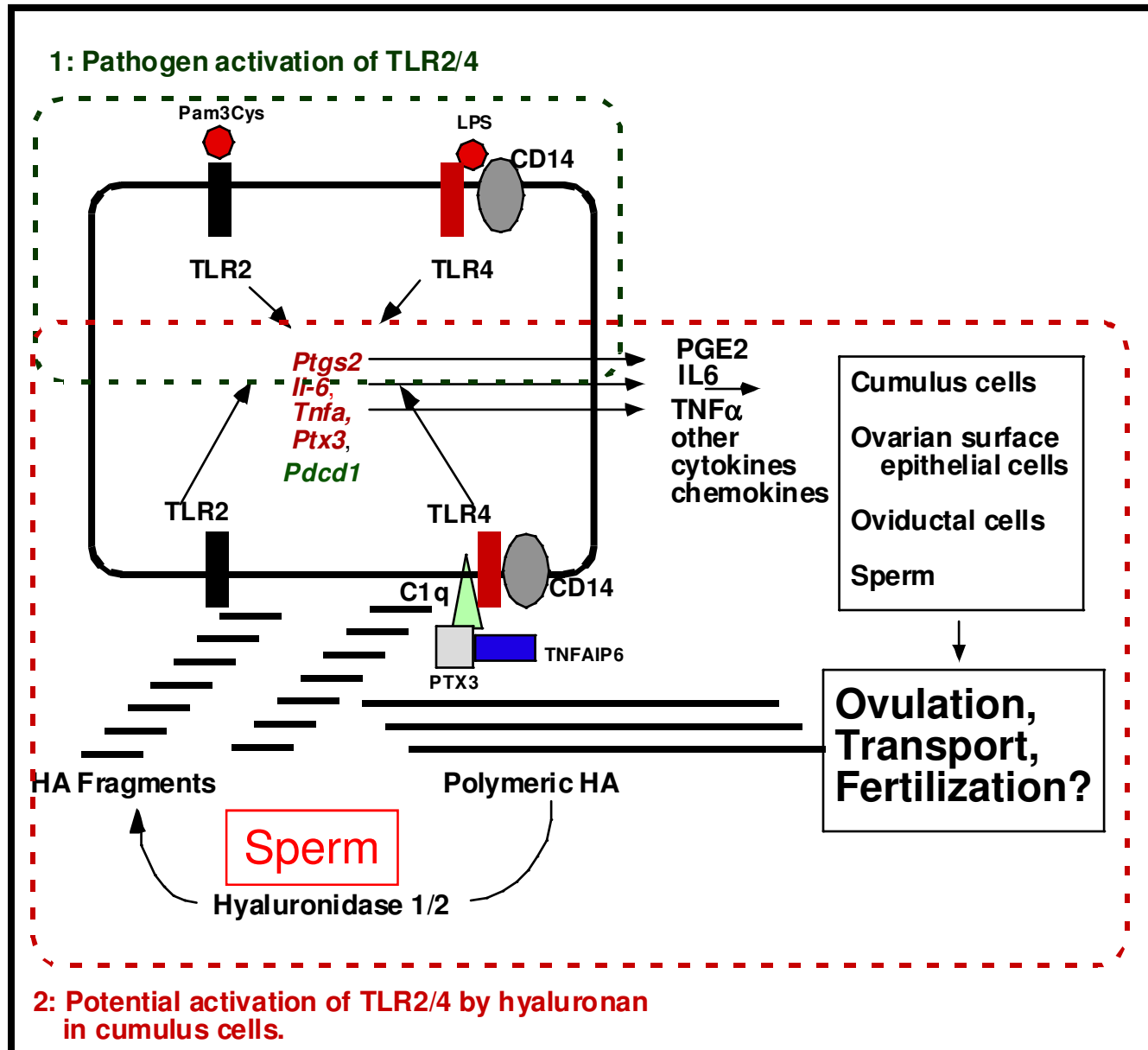


These cytokines are released from cumulus cells and granulosa cells by a progesterone receptor-dependent mechanism that involves the induction of the secretory vesicle protein SNAP25



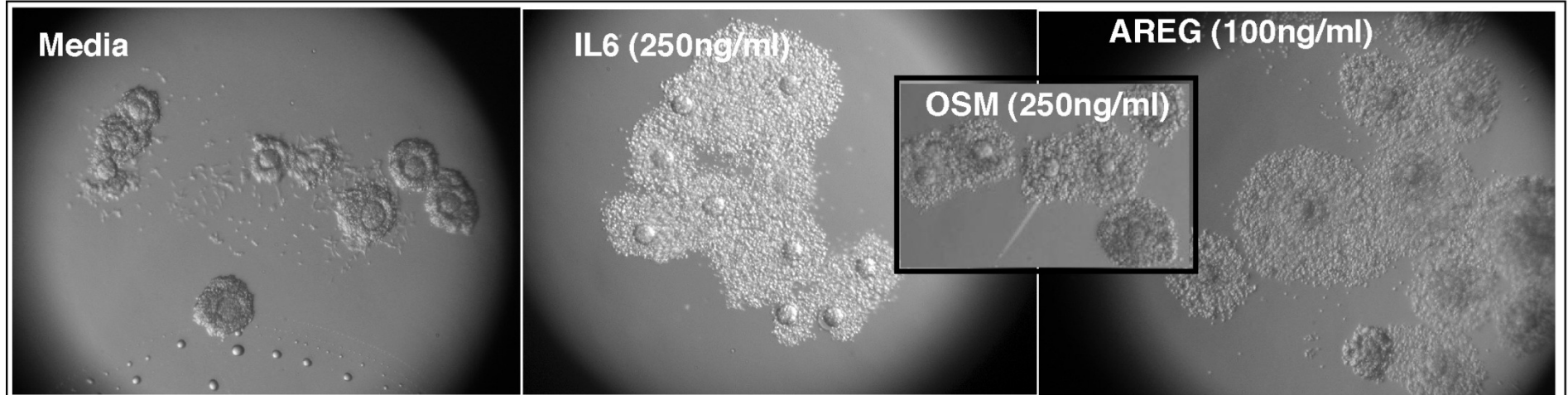
	free	FSH+AREG		Forskolin+PMA	
		-	RU486	-	RU486
IL-6	2.31+/-1.15	55.67+/-11.34*	22.15+/-3.22 [#]	168.13+/-15.41**	122.33+/-9.55 ^{##}
IL-9	0.98+/-0.20	5.35+/-1.69*	2.05+/-0.82 [#]	4.83+/-0.25**	1.91+/-0.72 ^{##}
IL-17	0.54+/-0.06	5.28+/-0.95*	4.92+/-0.55	11.23+/-1.84**	8.25+/-0.51 ^{##}
KC	33.15+/-4.64	51.60+/-14.46	35.22+/-3.51	81.85+/-12.28**	60.22+/-5.15 ^{##}
RANTES/ CCL5	1.25+/-0.68	1.16+/-0.13	1.61+/-0.55	2.11+/-0.83	1.90+/-0.31

Potential roles of the TLR pathway in the female reproductive tract before and after ovulation: pathogens and matrix factors

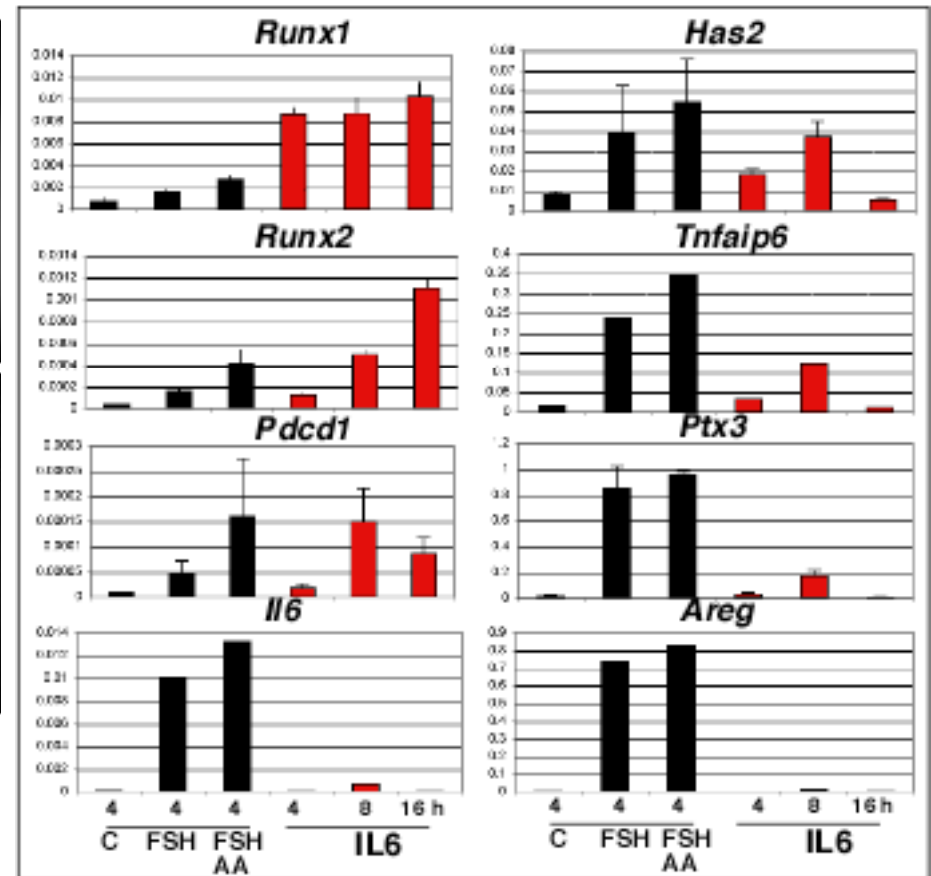
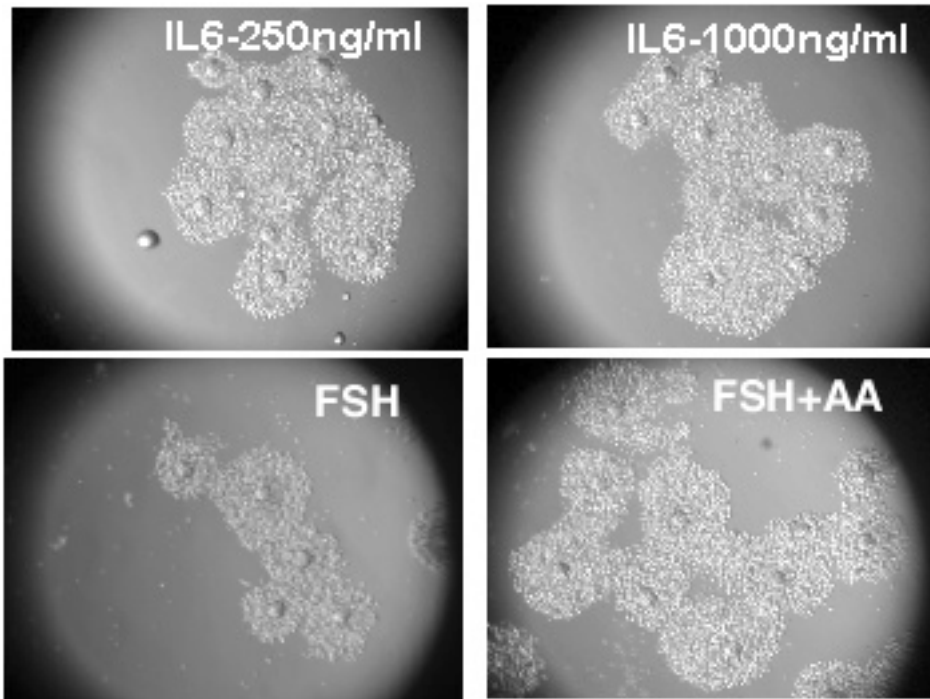


What are the functions of IL6 and other cytokines?

IL6 and OSM induce expansion of mouse COCs and oocyte maturation in culture



IL6 induces COC expansion and matrix-associated genes but not *Il6* or *Areg* in cultured COCs



IL6 also enhances IVF success and embryo viability.

What are some other potential roles of these cytokines and chemokines?

Sperm have receptors for cytokines and chemokines that impact sperm motility to enhance fertilization.

Sperm also express functional receptors for TLR2/4 that appear to impact sperm motility, viability and fertilization capacity.

Many infertile men and male domestic animals have infections within the genital tract that reduces fertility and impairs sperm function (causes apoptosis) in IVF protocols and in long-term sperm storage.

Antibiotics that block the action of LPS vastly improve sperm functions and viability in these infertile IVF protocols.

(Shimada *et al* Development, 2008 and personal communication)

Activation of TLR4/2 has also been linked recently to obesity, insulin resistance and diabetes via sensing and responding to FFA.

Long chain fatty acids (palmitate) can activate TLR4 leading to production of pro-inflammatory cytokines via NFkB activation in adipocytes, hypothalamic cells and muscle cells.

Thus, inappropriate induction and activation of TLR4 by abnormal metabolic products may alter cellular homeostatic metabolic processes, leading to impaired functions of many tissues.

Loss of TLR2/4 protects mice from the effects of diet induced obesity.

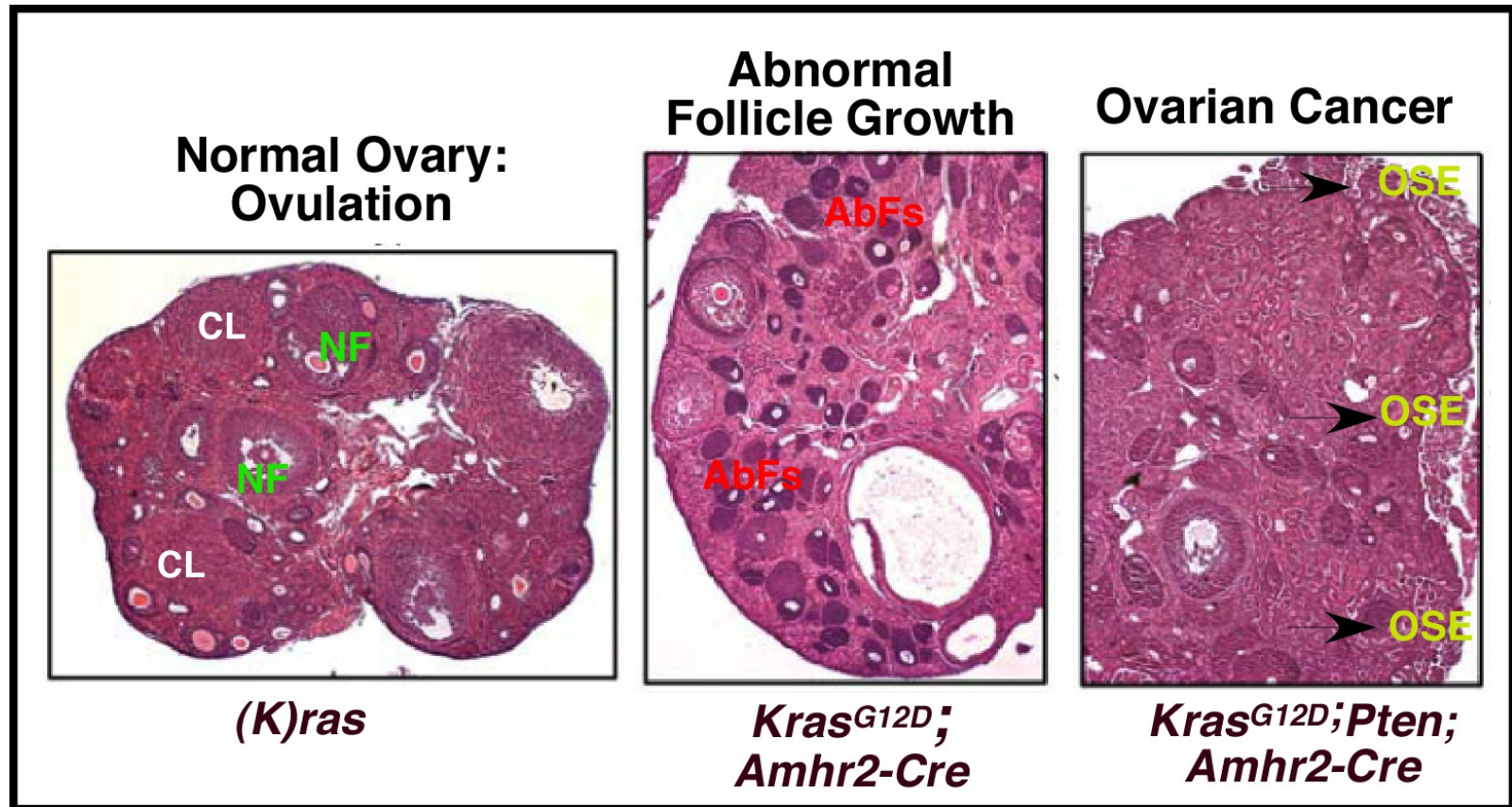
(Vitseva et al, Obesity, 2008; Milanski et al, J Neuroscience, 2009; Reyna et al, Diabetes, 2008)

In the ovary, this might translate to PCOS and insulin resistance.

Macrophages **remove** “non-self” (bacteria;LPS) or “altered self” (apoptotic cells) via scavenger receptors (**CD36, SCARB1/II**) that are induced by cytokines, such as IL6.

Do cumulus cells or granulosa cells exhibit similar functions?

There are also pathological as well as physiological functions of RAS, ERK1/2 and inflammatory molecules in the ovary.



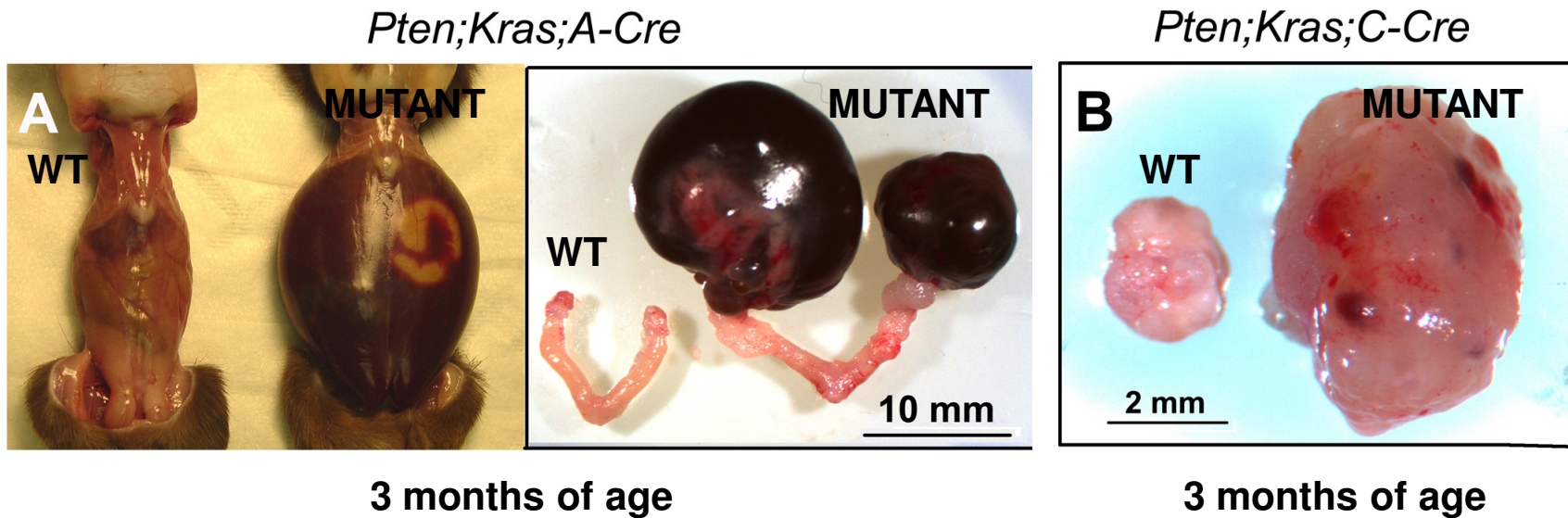
Obligatory for LH-induction of ovulation and luteinization, terminal differentiation
Fan *et al*, Science, 2009

Premature activation causes granulosa cell cycle arrest and abnormal follicle growth.
Fan *et al*, Development, 2008

Pten/Kras mutant OSE cells develop into serous adenocarcinomas but not GCTs.
Fan *et al*, Cancer Res, 2009

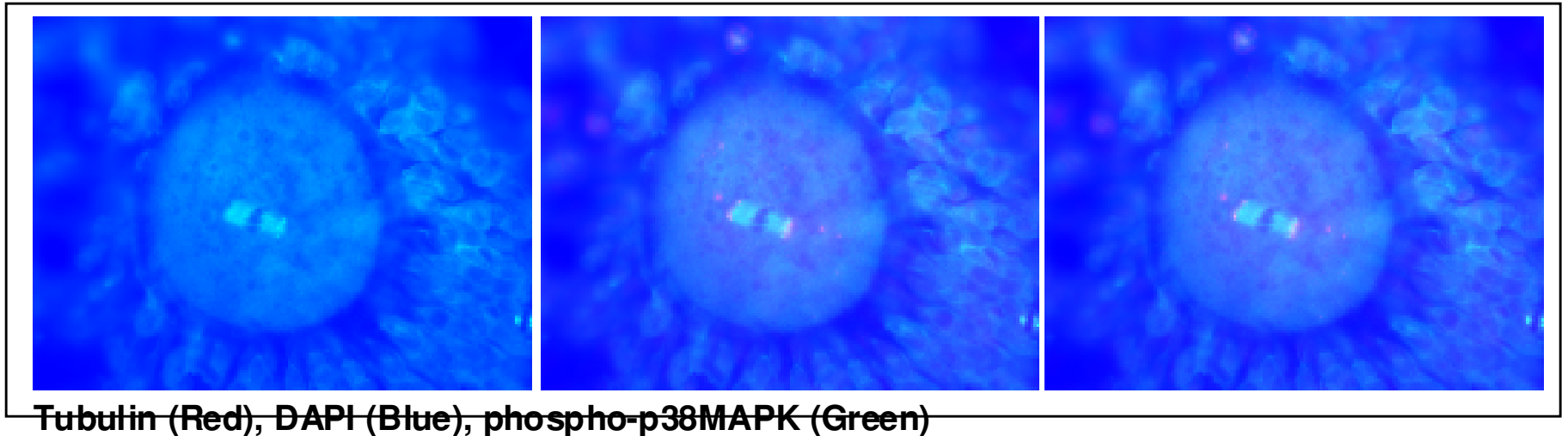
Ovarian surface epithelial (OSE) cell tumors
form in the *Pten;Kras;Amhr2-Cre* double mutant ovaries.

Tumors do not form in the *Pten;Kras;Cyp19-Cre* double mutant ovaries.



Therefore, **epithelial cells** respond to the *Pten;Kras* mutations in a manner that is completely different from the response of **granulosa cells** to the same oncogenic insults. A big question is why.

Clinical Relevance



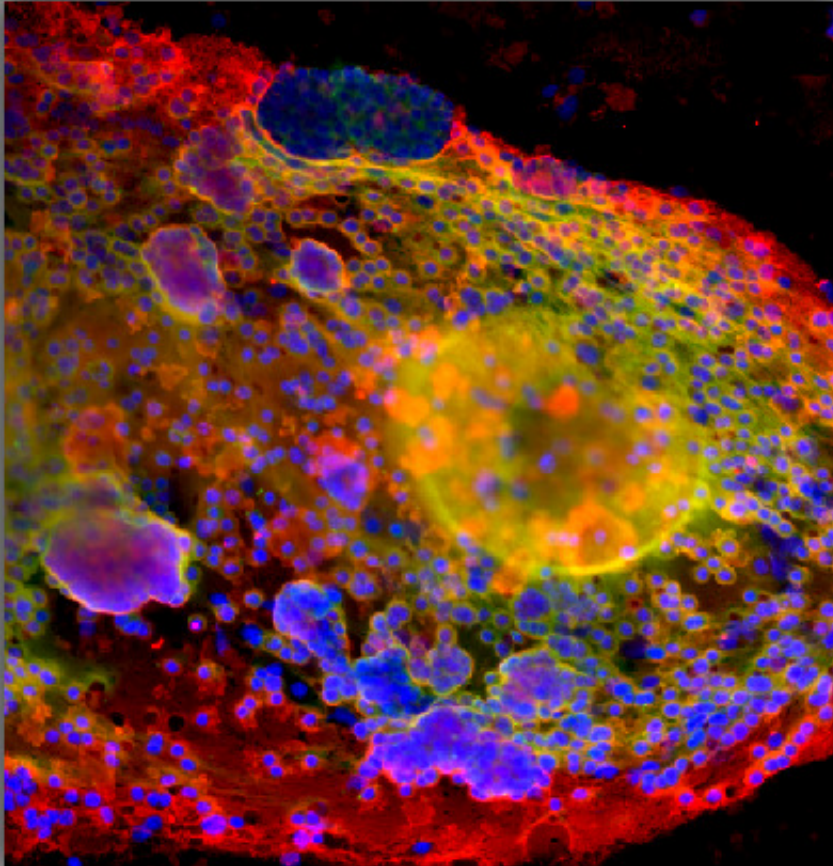
Role of EGF-like factors, RAS, ERK1/2 and IL6 in COCs, cumulus cells and oocytes as well as in growing follicles (POF) and ovarian cancer.

Role of TLR receptor pathways and HA in cumulus cells and sperm during fertilization and in infertile men.

New markers of oocyte quality and cumulus cell function/viability

Do chronic infections, such as **endometriosis** and autoimmune diseases contribute to PCOS and other abnormal ovarian functions, infertility and poor oocyte quality ---- **cancer**? Are the TLRs present on non-immune cells also involved in “sensing” and responding to abnormal cues derived from metabolic imbalance or malignant cells?

Acknowledgements



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Derek Boerboom
Darryl Russel
Rebecca Robker
Scott Ochsner

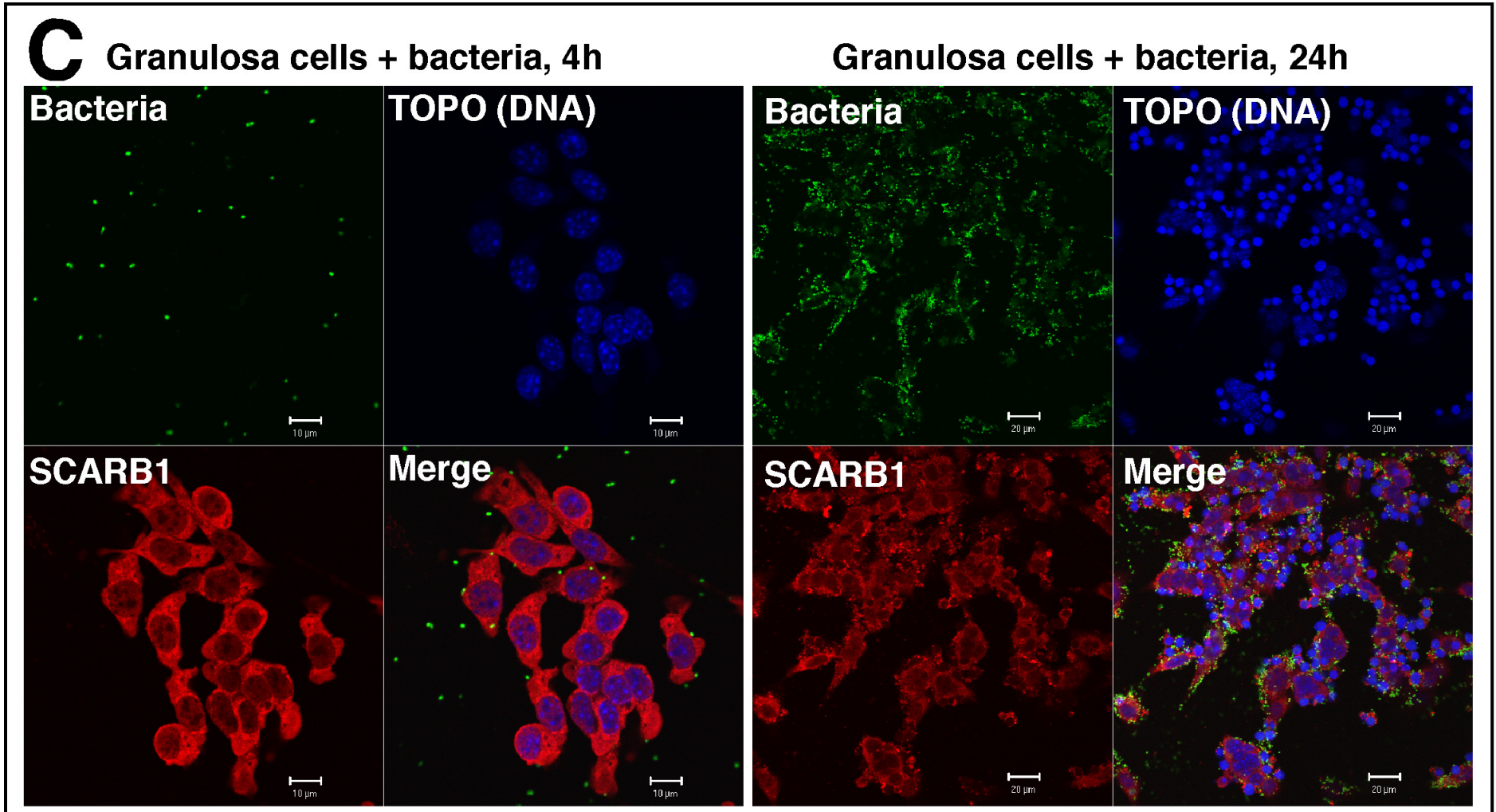
Microarray Core

Lisa White

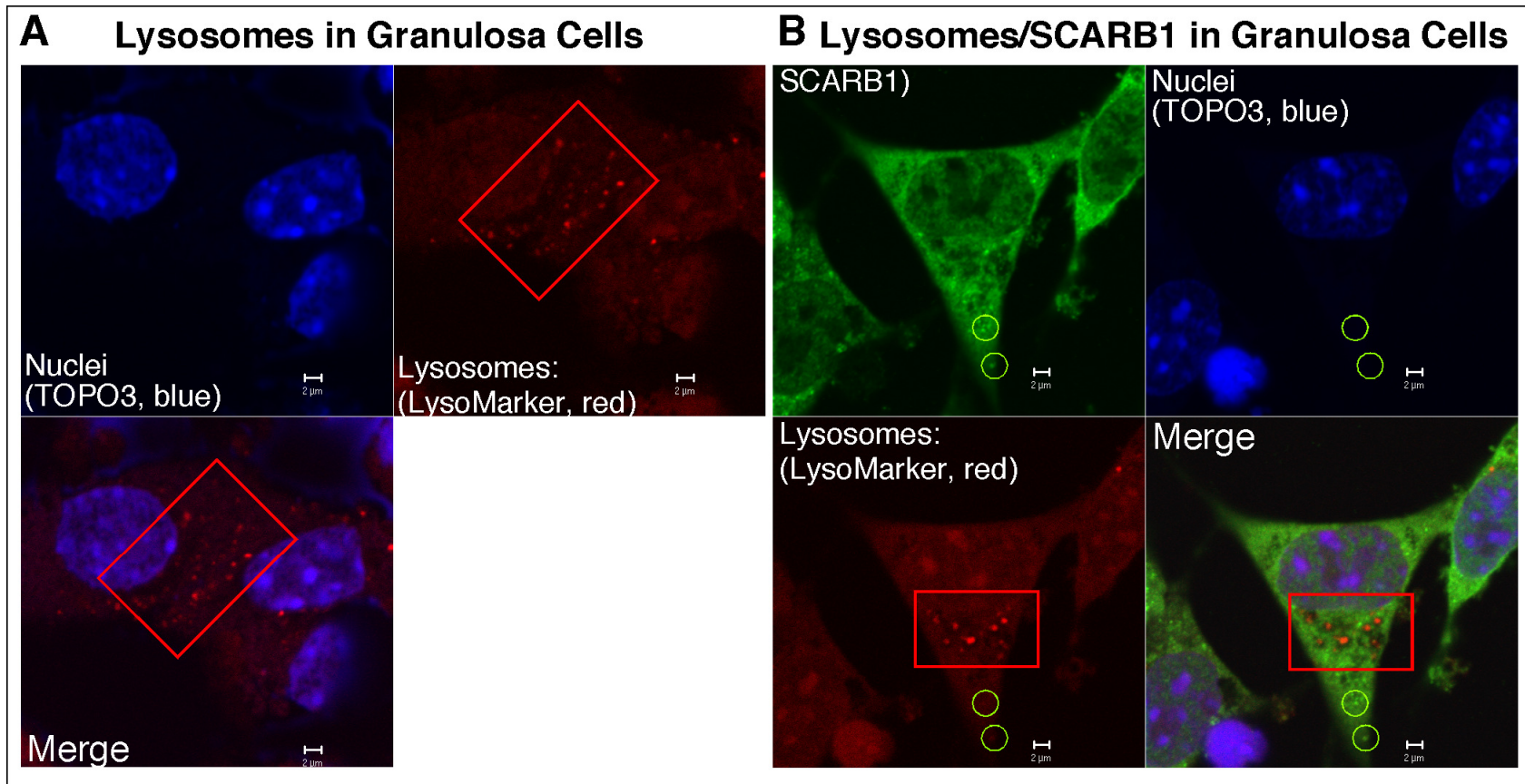
Colleagues

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Jan Gossen, Tyler Jacks, Jan Gossen
Peter Johnson, Esta Sterneck
Steve Hendrick, Gilles Pages,
Jacques Pouyssegur

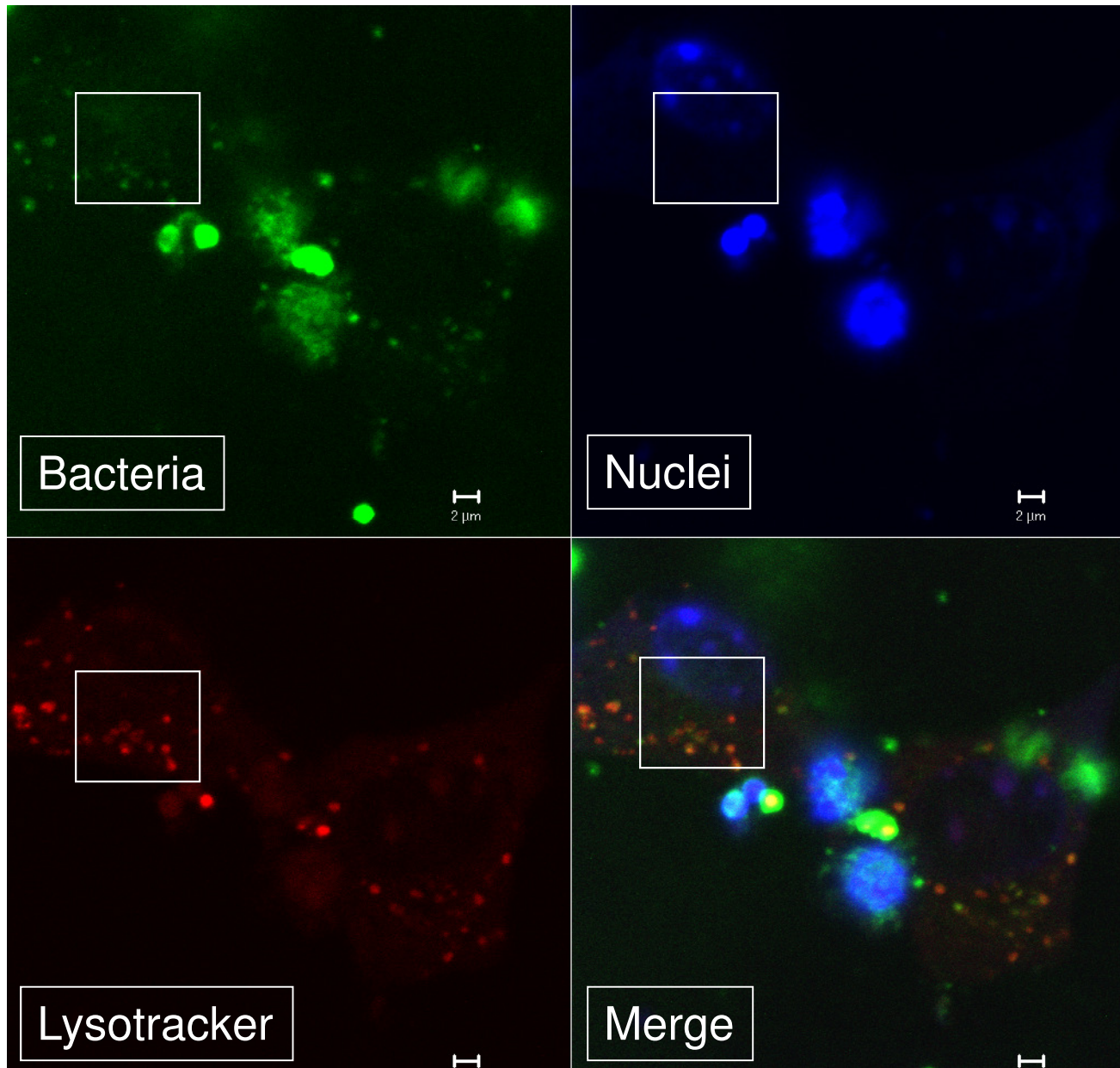
Bacteria attach to SCARB1 positive granulosa cells after 24h in culture



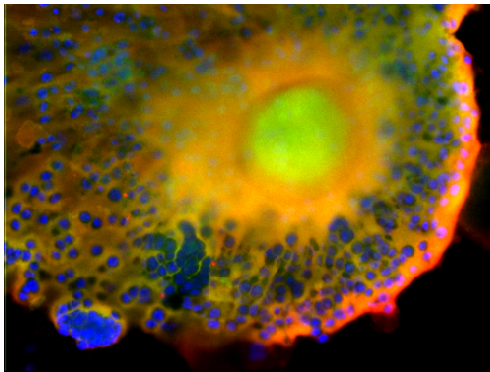
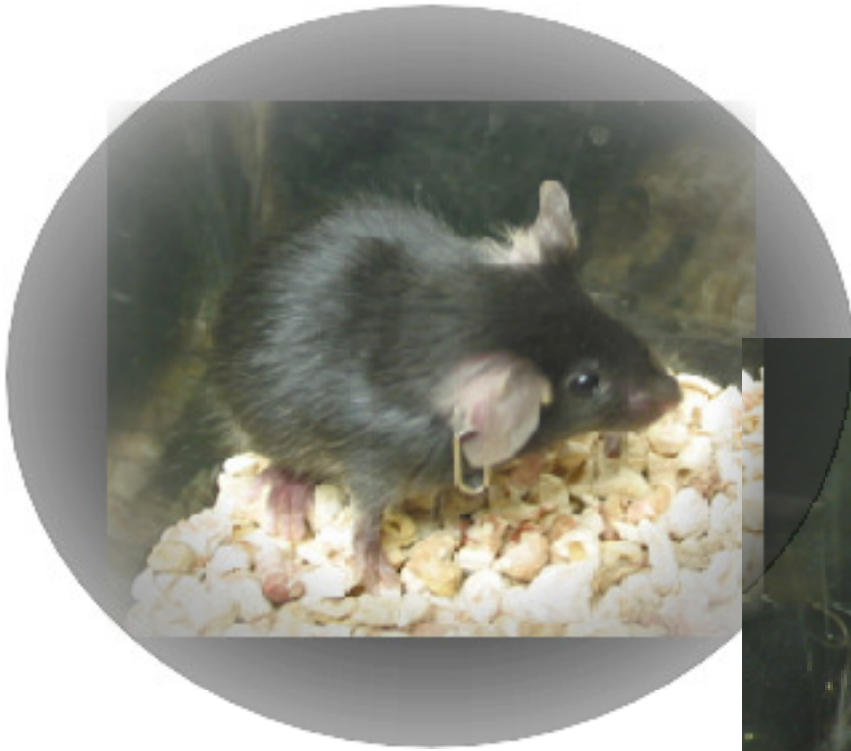
Localization of lysosomes (red) and SCARB1 (green) in granulosa cells



Bacteria co-localize to lysosomes in granulosa cells

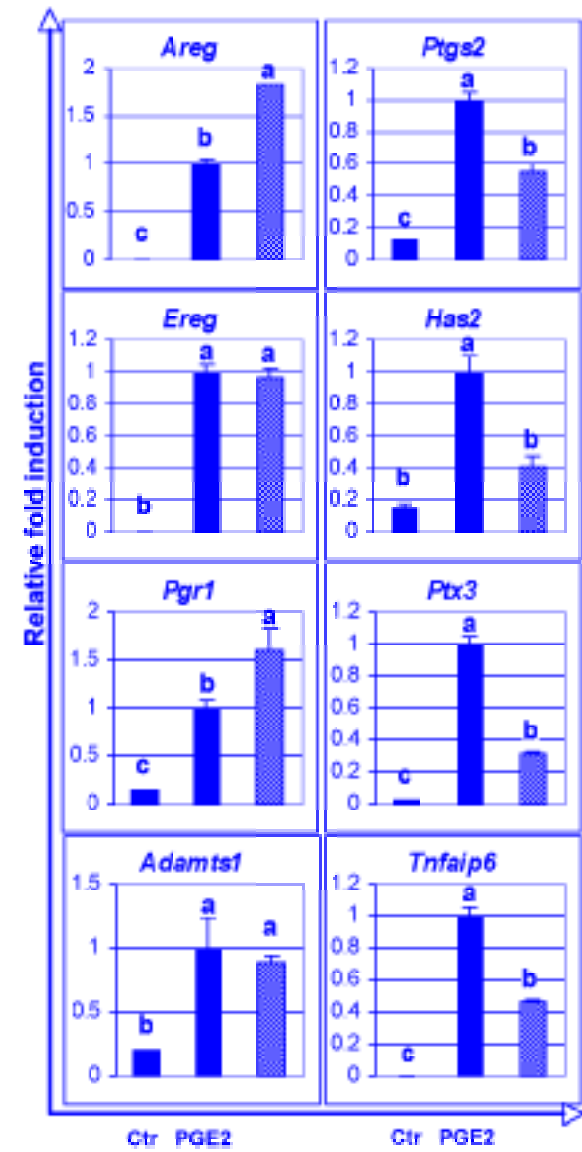
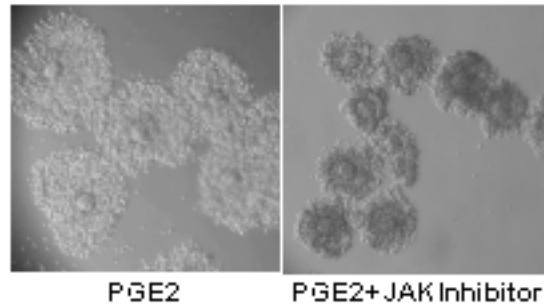


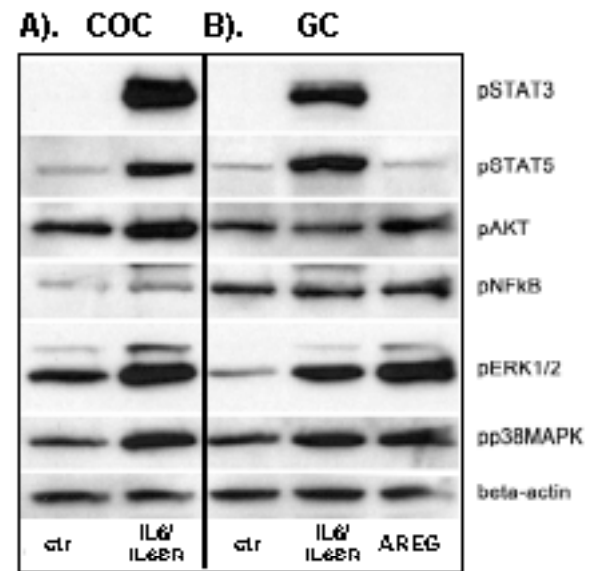
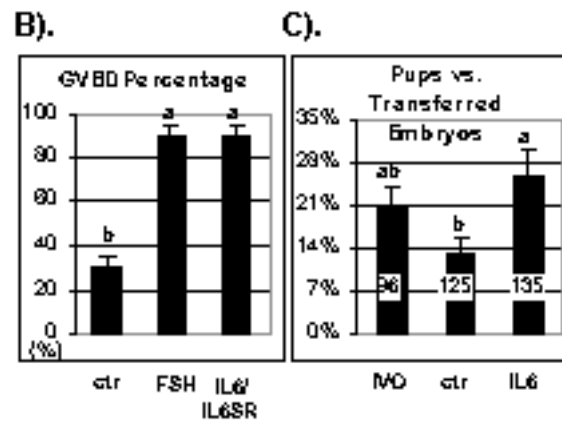
Heroes of tomorrow



**What are the functions of this vast repertoire of immune-like genes?
What is the embryonic derivation of cumulus cells?**

A).





What are the physiological roles of the TLR receptor pathway in granulosa cells and cumulus cells?

Do matrix molecules regulate these receptors?

Does hyaluronan act as a ligand like LPS?

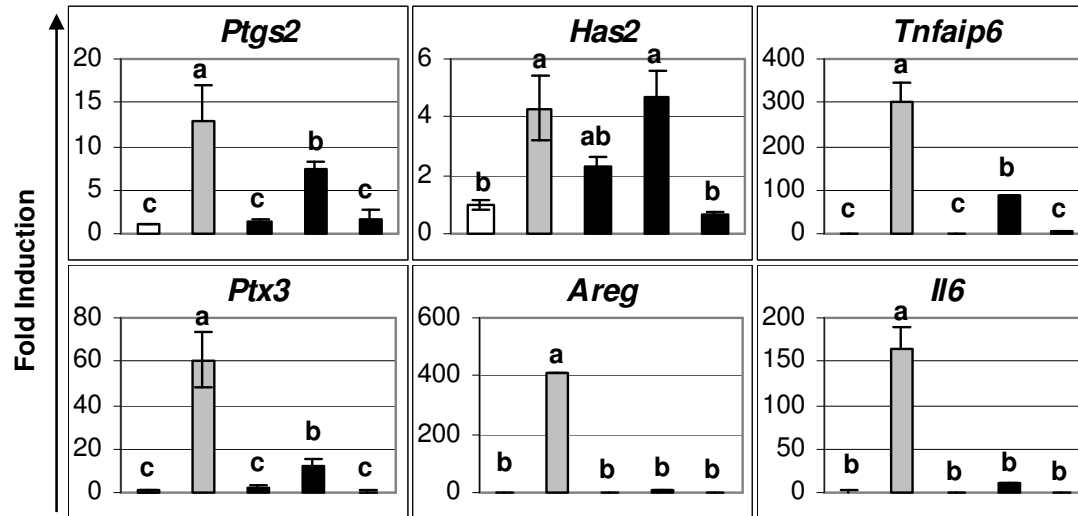
Is this pathway linked to the production and action of cytokines, such as IL6?

Is the TLR receptor pathway also functional in sperm?

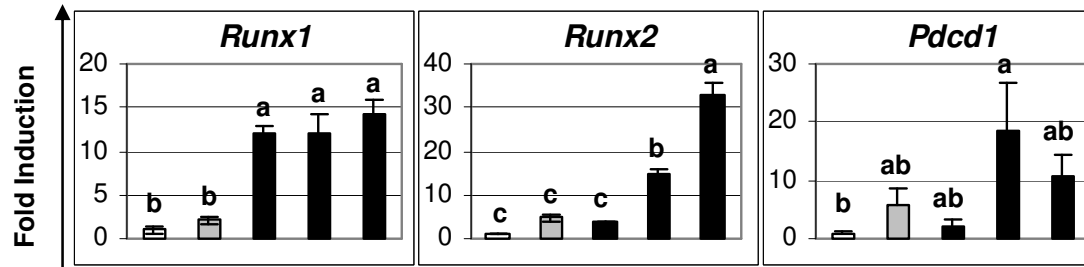
Does the TLR pathway mediate infertility (sperm dysfunction) in men with bacterial infections of the reproductive tract?

IL6 regulates matrix and immune-related genes in COCs

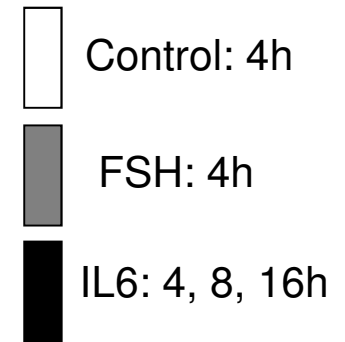
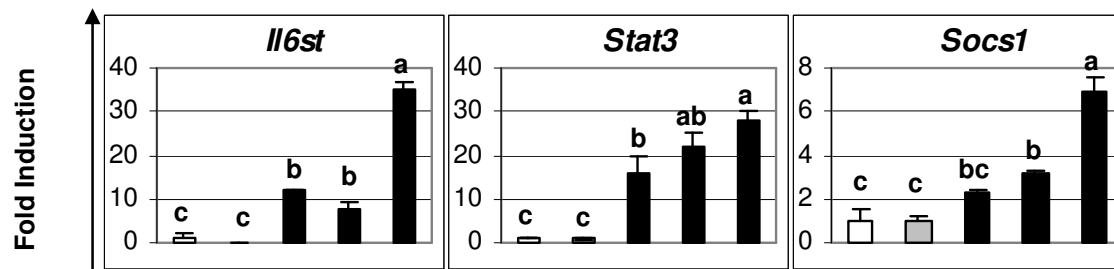
A). COC expansion genes



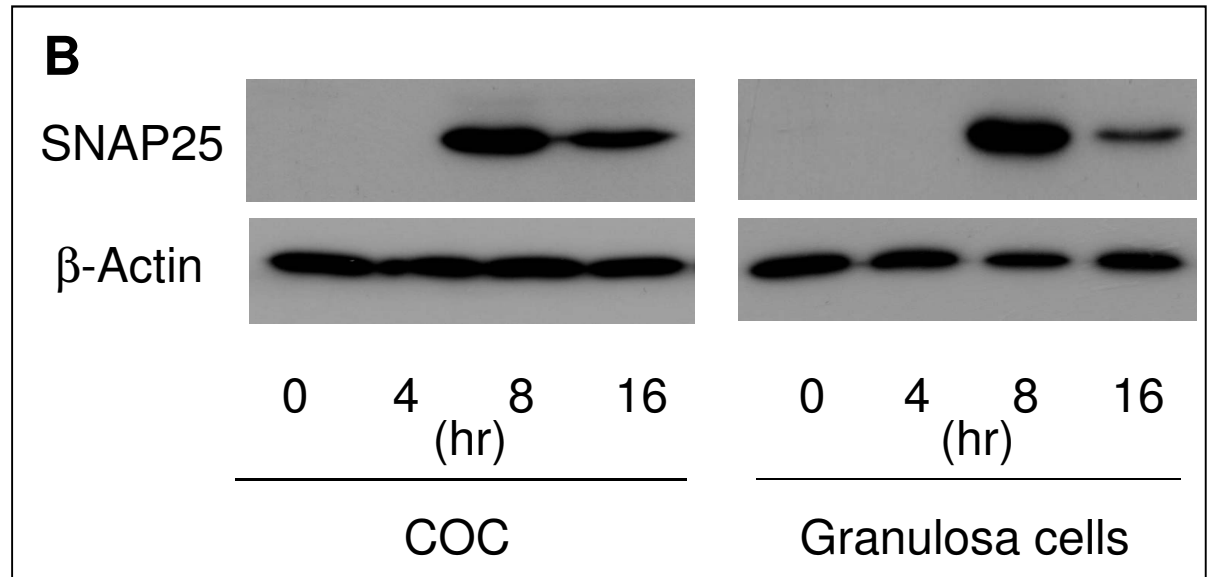
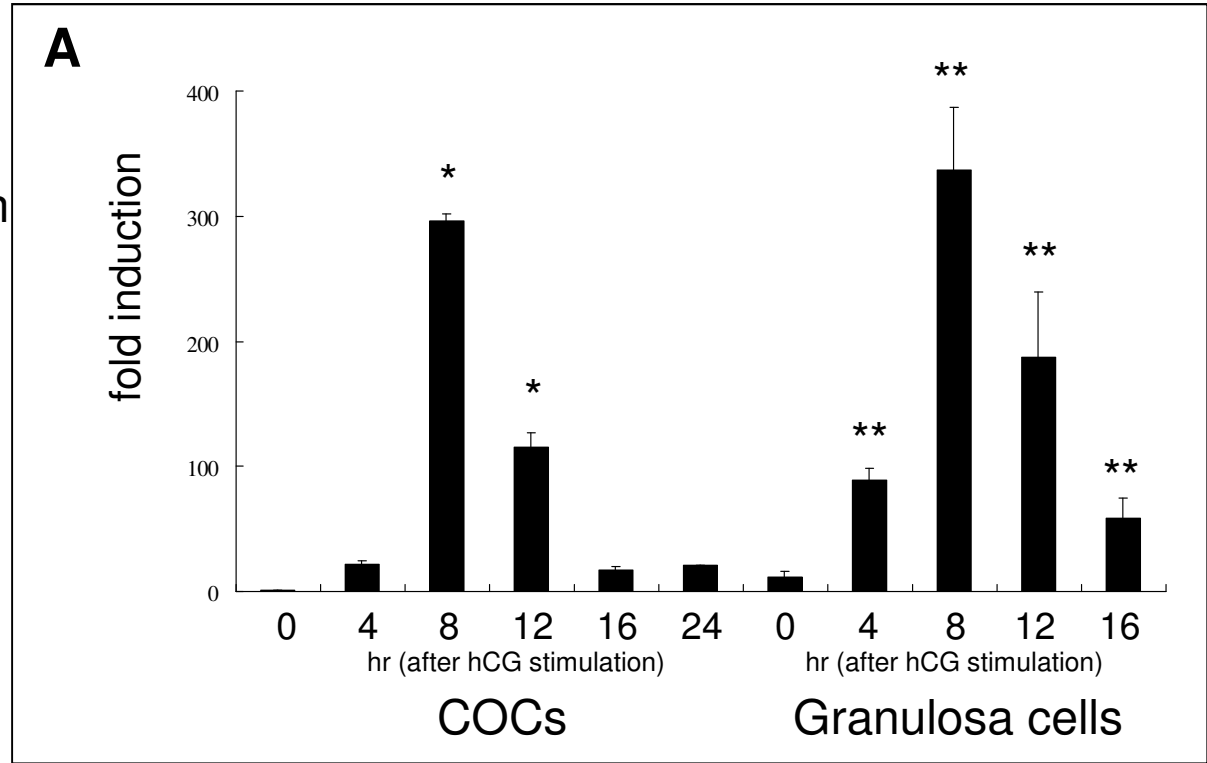
B). Immune related genes



C). IL6/JAK/STAT pathway genes



Snap mRNA and protein are induced in COCs and GC during the ovulation process

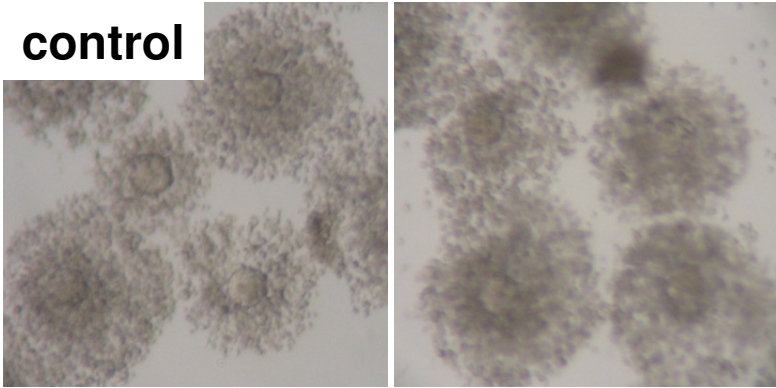


QuickTime™ and a
Video decompressor
are needed to see this picture.

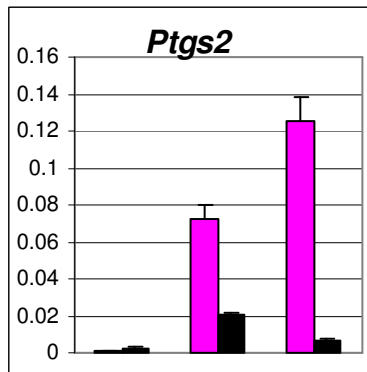
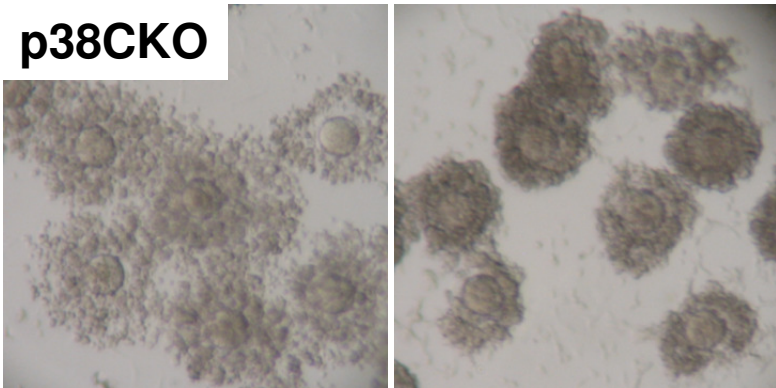
AREG

FSH

control



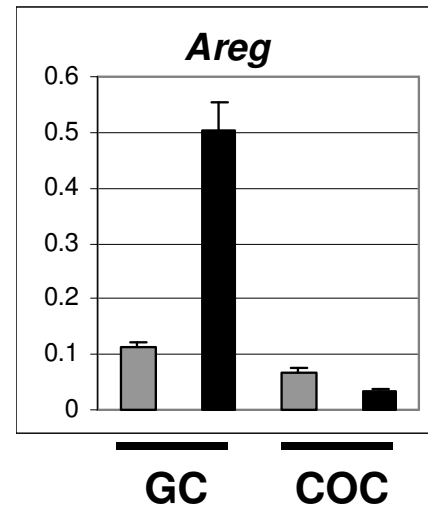
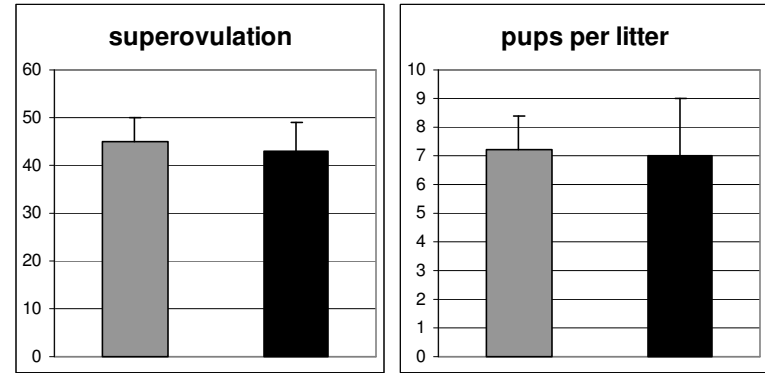
p38CKO



0h 1.5h 4h

■ p38CKO

p38MAPK CKO mice are fertile



Fertilization is compromised in the presence of TLR2/4 and CCL5 neutralizing antibodies that impair the release of cytokines/chemokines from cumulus cells that enhance sperm capacitation.

